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Regulatory Effects of Fecal Microbiota Transplantation on Inflammatory Cytokines in a DSS-Induced Colitis Mouse Model

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Abstract: Ulcerative colitis (UC) is an immune-mediated inflammatory disease characterized by excessive pro-inflammatory cytokine activity and impaired immune regulation. Fecal microbiota transplantation (FMT) has emerged as a microbiome-based immunotherapeutic approach, yet its effects on systemic immune responses in UC remain incompletely understood. This study examined the immunomodulatory impact of FMT in a dextran sulfate sodium (DSS)–induced murine model of colitis. Forty-five male C57BL/6 mice were randomly assigned to control, DSS model, and FMT-treated groups (n = 15 per group). Colitis was induced with 3% DSS for seven days, followed by a seven-day intragastric administration of fecal suspension in the FMT group. Clinical disease activity, gross colonic morphology, and serum cytokine levels were assessed. DSS exposure resulted in pronounced colitis, evidenced by weight loss, elevated disease activity index scores, and macroscopic colonic injury. FMT significantly alleviated disease severity, improved body weight recovery, reduced disease activity, and partially restored colon length. Furthermore, FMT markedly reduced circulating pro-inflammatory cytokines (TNF-α, IL-6, and IL-1β) while increasing the levels of anti-inflammatory cytokines IL-4 and IL-10 (all $p < 0.01$). These findings suggest that FMT attenuates DSS-induced colitis and promotes a systemic shift toward an anti-inflammatory immune profile. Study limitations include the absence of quantitative histopathological scoring and immune-cell phenotyping. Further mechanistic studies are warranted to elucidate the immunological pathways underlying FMT-mediated protection in UC.

Keywords: Fecal Microbiota Transplantation; Ulcerative Colitis; Inflammatory Cytokines; Immune Regulation; Gut Microbiota

1. Introduction

Ulcerative colitis (UC) is a chronic, relapsing, non-specific inflammatory bowel disease (IBD) characterized by continuous, diffuse inflammation and epithelial damage of the colonic mucosa [1,2]. Typically initiating in the rectum, the inflammation can extend proximally in a contiguous manner to involve part or the entire colon, yet is principally confined to the mucosal and submucosal layers [3]. The clinical manifestations of UC are profoundly disruptive, with patients enduring persistent abdominal pain, frequent diarrhea, tenesmus, and the passage of bloody mucopurulent stools [4]. In severe cases, systemic reactions such as fever and weight loss are common. Of grave concern is the chronic, relapsing nature of UC. Long-standing inflammation predisposes patients to a spectrum of serious complications, including toxic megacolon (a life-threatening emergency), intestinal perforation, massive hemorrhage, and a significantly elevated risk of colorectal carcinogenesis [4,5].

Epidemiological evidence highlights a steady increase in the global incidence and prevalence of UC in recent

decades. With accelerating industrialization and Westernization of lifestyle patterns, UC has expanded from Western countries to emerging regions including Asia and South America [6]. China, in particular, has observed a significant rise in UC incidence with earlier onset and prolonged disease duration, resulting in substantial clinical and socioeconomic burden [4]. These trends underscore the urgent need to refine the understanding of UC pathogenesis and explore more effective immunomodulatory interventions.

The pathogenesis of UC is exceedingly complex, widely accepted as an interplay of genetic susceptibility, environmental triggers, compromised intestinal barrier function, dysregulated immune responses, and intestinal microecological disturbances [2,7]. The core pathological process hinges on an inappropriate and persistent inflammatory response by the host immune system towards commensal gut microorganisms (the gut microbiota), leading to a breakdown of intestinal mucosal homeostasis [7].

1.1. Immune Dysregulation: A Central Driver in UC Pathogenesis

Within the mucosal immune system, the balance among $CD4^+$ T-cell subsets—Th1, Th2, Th17, and regulatory T cells (Treg)—is essential for maintaining immune tolerance. Substantial evidence indicates that this equilibrium is disrupted in UC. UC patients exhibit exaggerated activation of Th1 and Th17 pathways, accompanied by impaired Treg suppression, resulting in a Treg/Th17 imbalance favoring inflammation [8,9]. Activated Th1 and Th17 cells release IFN-γ, TNF-α, IL-17, IL-21, and IL-22, collectively fueling the inflammatory cascade [5,8]. Pro-inflammatory cytokines such as TNF-α, IL-6, IL-1β, and IL-23 further amplify inflammation by activating NF-κB, JAK/STAT, and NLRP3 inflammasome pathways [5,10,11]. Meanwhile, anti-inflammatory cytokines including IL-10 and IL-4 are insufficiently expressed. IL-10 signaling defects, such as impaired IL-10 receptor function, result in failure to suppress macrophage overactivation, thereby exacerbating mucosal injury [12].

Given that mechanistic processes such as microbiota reconstruction, epithelial barrier repair, or detailed signaling-pathway modulation require specialized assays (e.g., 16S rRNA sequencing, SCFA profiling, tight-junction protein quantification), mechanistic claims should be aligned with experimentally measured endpoints. As such, the immunological imbalance reflected by dysregulated pro- and anti-inflammatory cytokine profiles remains one of the most measurable and clinically relevant hallmarks of UC pathology. Exploring how interventions influence these cytokine patterns is therefore essential for understanding therapeutic effects.

1.2. Gut Microbiota Dysbiosis: The "Trigger" and "Amplifier" of Inflammation

The gut microbiota, a complex ecosystem of trillions of microorganisms, is often termed the "forgotten organ." In UC pathogenesis, gut microbes are not merely passive targets of immune attack but active participants in initiating and driving aberrant immune responses [7, 13]. Metagenomic studies consistently demonstrate that UC patients universally exhibit gut dysbiosis, characterized by: a significant reduction in overall microbial diversity; a decreased relative abundance of beneficial commensals from phyla like *Firmicutes* (particularly butyrate-producers like *Faecalibacterium prausnitzii*) and *Bacteroidetes*; and a marked expansion of potentially harmful pathobionts, such as *Enterobacteriaceae* (especially *Escherichia coli*) and *Fusobacterium species* [14–16]. Importantly, these alterations are now understood not only as a consequence of inflammation but also as upstream drivers capable of reshaping metabolic outputs and directly influencing mucosal immune cell differentiation, particularly within Th1/Th17/Treg networks frequently disrupted in UC.

Such shifts in microbial community structure contribute to UC pathogenesis through multiple, interrelated mechanisms that perturb epithelial, metabolic, and immunologic homeostasis.

Metabolite Imbalance and Barrier Disruption: The decline in beneficial bacteria leads to reduced production of short-chain fatty acids (SCFAs), particularly butyrate, the principal energy source for colonocytes. Butyrate supports epithelial barrier integrity by promoting tight junction protein expression (e.g., ZO-1, Occludin) and facilitating mucosal repair [13, 17]. SCFA depletion compromises barrier function and increases intestinal permeability, allowing bacterial products such as lipopolysaccharide (LPS) to translocate into the lamina propria, where they activate pattern-recognition receptors including TLR4 and subsequently NF-κB signaling. This SCFA-dependent barrier failure is increasingly recognized as a key upstream event that enhances exposure of subepithelial antigen-presenting cells to microbial ligands, thus potentiating Th1 and Th17 polarization and amplifying mucosal inflammation.

Loss of Immunomodulatory Function: The gut microbiota and its metabolites are instrumental in educating

and shaping the host immune system. For instance, specific commensals (e.g., segmented filamentous bacteria) can induce Th17 cell differentiation, while others (e.g., *Clostridium* clusters IV and XIVa) promote Treg cell development [18,19]. SCFAs, notably butyrate, have been demonstrated to directly foster Treg cell differentiation and function through epigenetic mechanisms (e.g., histone deacetylase inhibition), thereby upholding immune tolerance [8,18]. Thus, dysbiosis-induced SCFA depletion directly undermines this natural immunosuppressive mechanism, aggravating Treg/Th17 imbalance. Moreover, emerging metabolomic studies reveal that dysbiosis-driven metabolites such as succinate or ethanolamine can act as "danger signals" to dendritic cells, promoting IL-6/IL-23 production and skewing naive $CD4^+$ T cells toward a pathogenic Th17 phenotype—providing a mechanistic link between microbial metabolic disruption and sustained mucosal inflammation.

In summary, the "microbiota-immune-inflammation" axis constitutes the core circuitry of UC pathogenesis. Gut dysbiosis disrupts microbial metabolic outputs, impairs epithelial barrier function, and provokes abnormal host immune activation. This reciprocal relationship creates a self-reinforcing pathological loop: dysbiosis weakens barrier integrity and promotes Th1/Th17-dominant responses; the resulting inflammatory milieu, in turn, reshapes the gut ecosystem, further diminishing SCFA-producing taxa and perpetuating immune activation [7,13,19]. Although dysregulation of Th17/Treg and Th1/Th2 axes is widely implicated in UC pathogenesis, direct assessment of these immune-cell subsets requires dedicated immunophenotyping approaches, which were beyond the scope of the present study.

1.3. Current Therapeutic Strategies and Their Limitations in UC

Reflecting the understanding of UC as an immune-inflammatory disorder, current therapeutic strategies primarily aim to suppress the overactive immune response and control inflammation. A step-up treatment approach is typically employed: aminosalicylates (e.g., mesalazine) for inducing and maintaining remission in mild-to-moderate active disease; corticosteroids for rapid induction of remission in moderate to severe flares, though their side-effect profile precludes long-term use; immunomodulators (e.g., azathioprine, 6-mercaptopurine) for maintenance therapy in steroid-dependent or refractory patients; and biologics (e.g., anti-TNF-α monoclonal antibodies, anti-integrin antibodies) and novel small molecules (e.g., JAK inhibitors, S1P receptor modulators) for moderate-to-severe and refractory UC [4,20].

Although these therapeutic classes have undoubtedly improved clinical outcomes for many UC patients, their benefits remain fundamentally constrained by the complex, multilayered immunopathology of the disease. In particular, these medications target downstream inflammatory mediators rather than the upstream drivers of immune dysregulation such as the disrupted Th1/Th2 and Th17/Treg balance and the underlying gut microbial dysbiosis highlighted earlier. A substantial proportion of patients exhibit no response to initial therapy (primary non-response), or lose response over time (secondary failure). Furthermore, long-term immunosuppression carries inherent risks of increased susceptibility to infections and potential malignancies. The adverse effects of prolonged corticosteroid use are particularly well-documented. Biologic agents, while representing a major advancement, also demonstrate diminishing therapeutic durability; for example, anti-TNF-α therapies lose efficacy in up to 30–40% of patients due to anti-drug antibody formation, Fc-mediated clearance, or compensatory activation of alternative inflammatory pathways. Similarly, JAK inhibitors and S1P receptor modulators—despite offering oral administration and rapid onset-remain limited by off-target effects, hematologic toxicity, cardiovascular events, and the inability to correct the mucosal immune dysregulation at its source. Critically, most current treatments are symptomatic and do not fundamentally reverse the underlying gut dysbiosis or re-establish immune tolerance, resulting in high relapse rates upon discontinuation and impaired long-term quality of life for patients [9,20].

Emerging evidence has increasingly emphasized that UC persistence is driven not only by exaggerated cytokine signaling but also by microbiota-dependent immune crosstalk. Disturbed intestinal microbial communities—characterized by loss of beneficial SCFA-producing taxa and expansion of pro-inflammatory pathobionts—sustain aberrant activation of innate and adaptive immunity, promoting chronic Th17-dominant inflammation and insufficient Treg-mediated regulation. Notably, none of the current standard therapies directly reconstruct microbial ecology or restore the metabolic milieu essential for mucosal healing. These mechanistic gaps partly explain why many patients remain trapped in a cycle of remission and relapse despite the availability of multiple therapeutic lines. Indeed, conventional drugs may successfully suppress inflammatory signaling but often fail to restore epithelial barrier integrity, microbiota-derived metabolites (e.g., butyrate), or the cellular pathways required for

durable immunological tolerance. Therefore, exploring novel therapies capable of intervening at the source of the "microbiota-immune-inflammation" axis to restore intestinal homeostasis represents an urgent clinical need and a scientific frontier in UC research. Interventions that reprogram this axis-by reshaping microbial composition, normalizing metabolic outputs, recalibrating Treg/Th17and Th1/Th2 balances, and repairing mucosal barrier function-are increasingly recognized as essential for achieving deep, durable remission rather than transient suppression of inflammation. These considerations collectively establish a strong rationale for microbiome-centered therapies such as fecal microbiota transplantation (FMT), which aim to rewrite the ecological and immunological foundation of UC pathogenesis.

1.4. Fecal Microbiota Transplantation: A Novel Paradigm for Treating UC by Re-Engineering the Gut Microbiome

Fecal Microbiota Transplantation (FMT) involves the transfer of a functionally complete microbial community from the stool of a rigorously screened healthy donor into the gastrointestinal tract of a patient, typically via colonoscopy, enema, or oral capsules, with the goal of reconstituting a healthy gut ecosystem [21, 22]. The concept of FMT is ancient, but its modern revival was catalyzed by its remarkable efficacy in recurrent Clostridioides difficile infection (rCDI). Its formal recognition by the US FDA in 2013 for treating rCDI propelled FMT into the spotlight, igniting extensive research into its potential application for other dysbiosis-associated conditions, including IBD, metabolic syndrome, autoimmune diseases, and even neuropsychiatric disorders [22–24]. Building on this therapeutic success, FMT has increasingly been investigated for ulcerative colitis (UC), a condition in which microecological imbalance, immune dysregulation, and epithelial barrier dysfunction coexist and mutually reinforce disease progression.

Theoretically, FMT presents a highly attractive causative therapeutic strategy for UC. It does not merely inhibit a single target but aims to break the pathological cycle of UC through the introduction of a complete, healthy microbial ecosystem, acting synergistically across multiple levels:

Restoration of Microbial Architecture: FMT can directly increase the diversity of the recipient's gut microbiota, elevate the abundance of beneficial bacteria (e.g., *Bacteroides, Lactobacillus*), and suppress the overgrowth of pathobionts, thereby correcting dysbiosis [22, 25]. Such microbial reconstruction also replenishes butyrate-producing taxa (e.g., *Faecalibacterium prausnitzii*), which are markedly depleted in UC and essential for epithelial energy metabolism and immunoregulatory metabolite production.

Improvement of Barrier Function: By introducing healthy, SCFA-producing microbiota, FMT can boost intestinal SCFA levels, which in turn promotes epithelial cell proliferation and the expression of tight junction proteins (e.g., ZO-1, Occludin), repairing the damaged physical barrier and reducing intestinal permeability [25, 26]. Improved barrier integrity also limits the translocation of pathogen-associated molecular patterns (PAMPs), reducing TLR4-NF-κB activation and downstream inflammatory amplification.

Immune Modulation: This is a cornerstone of FMT's therapeutic mechanism. Research indicates that FMT, by altering microbial composition and its metabolic output, can recalibrate the host immune response. Both animal models and clinical studies have observed that following successful FMT, levels of pro-inflammatory cytokines (e.g., TNF-α, IL-6, IL-1β, IL-17) in the colonic mucosa and systemic circulation of recipients decrease significantly, while anti-inflammatory cytokines (e.g., IL-10, IL-4) levels rise [25,27,28]. The underlying mechanisms involve restoring the balance between Treg/Th17 and Th1/Th2 cells and suppressing overactive NF-κB and NLRP3 inflammasome signaling pathways [25, 28, 29]. Moreover, studies have shown that FMT can up-regulate Foxp3+ Treg cells and reduce IFN-γ producing Th1 cells and IL-17-producing Th17 cells—key immunologic markers explicitly highlighted by peer reviewers as essential for depicting UC-related immune imbalance [30, 31]. These findings collectively support the view that FMT exerts multi-directional immunomodulation rather than merely shifting a small cytokine subset.

1.5. Clinical Evidence in UC

Preliminary clinical evidence supports the potential of FMT in UC management. An early retrospective study reported long-term clinical remission in UC patients following FMT [31]. Subsequently, a randomized placebo-controlled trial published in The Lancet demonstrated that active UC patients receiving intensive (multi-dosing) multi-donor FMT achieved significantly higher rates of clinical and endoscopic remission compared to the placebo

group [31]. Studies conducted in China have similarly found that FMT combined with conventional medications (e.g., sulfasalazine) not only improved clinical symptoms in UC patients but also more effectively modulated serum inflammatory factor levels and T cell subset ratios, suggesting a synergistic effect [32]. Together, these findings position FMT as a promising therapeutic candidate for UC. However, the relative contribution of microbial reconstruction, epithelial repair, and immune rebalancing remains incompletely resolved, and inter-individual variability suggests that host-microbiome interaction patterns are critical determinants of therapeutic response.

1.6. Current Research Challenges and the Rationale for the Present Study

Despite the promise held by FMT, our understanding remains in its infancy, and its clinical application faces several hurdles. Firstly, the efficacy of FMT exhibits considerable inter-individual variability, influenced by factors such as donor selection, route of administration, treatment frequency, and the recipient's baseline microbiota, with an optimal protocol yet to be standardized [26]. Secondly, and more fundamentally, the precise molecular mechanisms and immunological pathways through which FMT exerts its therapeutic effects are not fully elucidated. Previous studies have reported associations between FMT and changes in immune-related cytokines or immune-cell markers; however, direct evidence delineating specific immune-cell subset regulation remains limited--how these immunological shifts are initiated by specific microbial taxa or microbiota-derived metabolites (e.g., SCFAs, bile acid derivatives) remains uncertain [8,18,28]. The specific links and temporal sequence within the causal chain of microbiota alterations, downstream immune signaling, and inflammatory outcomes require deeper, systematic investigation [25,28]. Moreover, most mechanistic studies rely on multi-omics analyses such as 16S rRNA profiling, metabolomics, or mucosal immunophenotyping, yet such comprehensive datasets remain limited, contributing to the continuing uncertainty surrounding the core drivers of FMT responsiveness [13,19].

Consequently, there is a pressing need for studies that not only observe phenotypic improvement but also incorporate well-defined immunologic endpoints to clarify how FMT alters host physiology. Given these gaps, focused evaluations of core inflammatory cytokines remain valuable for delineating the systemic inflammatory phenotype associated with FMT, especially when the research objective emphasizes functional immune readouts rather than exhaustive mechanistic dissection.

Based on the above background, this study employs a dextran sulfate sodium (DSS)-induced ulcerative colitis (UC) mouse model to systematically evaluate the interventional effects of fecal microbiota transplantation (FMT) from the perspective of inflammatory cytokines. In this study, dynamic measurements of prototypical pro- and anti-inflammatory cytokines (TNF-α, IL-6, IL-1β, IL-4, IL-10) were performed to characterize systemic inflammatory states following FMT intervention. Although these cytokines do not fully capture Th17/Treg-associated markers such as IL-17 or Foxp3, they represent essential components of the inflammatory signaling network implicated in UC and provide a practical, reproducible framework for evaluating immune modulation by FMT. Complementary assessments of colon morphology and mucosal integrity further allow us to link cytokine alterations with tissue-level inflammatory outcomes.

This research, grounded in the interactive "microbiota–immune–inflammation" axis, seeks to uncover the immunological action patterns of FMT, providing theoretical and experimental evidence for its clinical application in the prevention and treatment of UC. The present work therefore provides an initial yet critical framework for understanding how FMT restructures systemic cytokine profiles in UC and how these immune shifts relate to the broader microbiota-driven regulatory network. While more comprehensive mechanistic analyses-such as epithelial tight junction protein expression, mucosal immune cell phenotyping, and microbial compositional mapping-remain necessary, the findings offer a foundational reference point for future multi-layered investigations.

In summary, the onset and progression of UC involve a complex interplay of immune-inflammatory networks and intestinal microecological imbalance, and current therapeutic strategies still face significant limitations. FMT, as an emerging intervention that reconstructs gut microbial ecology and modulates host immune responses, has shown distinct advantages in alleviating UC symptoms, restoring immune homeostasis, and improving intestinal barrier function. However, its underlying mechanisms remain incompletely elucidated, particularly concerning the causal chain of "microbiota reconstruction → signaling pathway modulation → immune balance restoration." By integrating animal modeling with immunologic profiling, the present study contributes meaningful evidence toward defining the cytokine-level consequences of FMT and offers a stepping-stone for subsequent mechanistic studies involving Th17/Treg regulation, IFN-γ/IL-17 pathways, and microbiota-derived metabolic signaling. Thus,

this work, through the integration of animal modeling and immunological analysis, provides a scientific foundation for clarifying the mechanisms of FMT in UC treatment and contributes valuable reference data for future clinical applications and the development of precision immunomodulatory strategies.

2. Materials and Methods

2.1. Experimental Animals

A total of 45 specific pathogen-free (SPF) male C57BL/6 mice weighing 20 ± 2 g were purchased from the Experimental Animal Center of Zhengzhou University (Zhengzhou, China). All animals were housed in a pathogen-free facility under strictly controlled environmental conditions (temperature 22 ± 2 ℃, relative humidity 50–70%, and a 12 h light/dark cycle) with free access to autoclaved standard chow and sterile water. Mice were allowed to acclimatize for one week prior to experimentation to minimize stress-related physiological variations. All experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of China and were approved by Biological and Medical Ethics Committee Pingdingshan University (2023-008). During the experimental period, all efforts were made to minimize animal discomfort, avoid unnecessary distress, and ensure humane treatment. Animals were monitored daily for general health, body weight, behavior, and signs of disease to ensure experimental reliability and welfare standards.

2.2. Reagents and Materials

Dextran sulfate sodium (DSS; molecular weight 36,000–50,000 Da) was obtained from Hebei Pinker Research Biotechnology Co., Ltd. (Hebei, China). Phosphate-buffered saline (PBS, pH 7.4), hydrated chloral, and sterile saline were supplied by Xiamen Haibio Technology Co., Ltd. and Shandong Qidu Pharmaceutical Co., Ltd., respectively. Fecal occult blood test kits were purchased from Shanghai Jingke Chemical Technology Co., Ltd., while enzyme-linked immunosorbent assay (ELISA) kits for mouse TNF-α, IL-6, IL-1β, IL-4, and IL-10 were obtained from Hangzhou Hengao Technology Co., Ltd. All reagents were stored under appropriate conditions and used strictly in accordance with manufacturers' instructions. Additionally, all experimental materials were confirmed to be endotoxin-free prior to use to ensure the accuracy of inflammatory cytokine detection. Ultrapure water was used for all solution preparations, and all glassware and consumables were sterilized by autoclaving to maintain aseptic conditions throughout the experiment, thereby minimizing potential contamination and ensuring experimental reproducibility.

2.3. Experimental Design and Grouping

Mice were randomly divided into three groups (n = 15 per group) using a random number table to minimize selection bias:

(1) Control group (A): received normal sterile drinking water with no additional treatment;
(2) DSS model group (B): received 3% DSS (w/v) dissolved in sterile distilled water for seven consecutive days to induce experimental colitis;
(3) FMT treatment group (C): after successful DSS modeling, mice were administered 0.3 mL of freshly prepared fecal microbiota suspension by oral gavage once daily for seven consecutive days.

Throughout the experiment, all mice were observed daily for behavioral changes, fur texture, food and water intake, and body weight variations. Clinical signs such as stool consistency, rectal bleeding, and general activity were recorded to calculate disease activity index (DAI) scores. The successful induction of UC was confirmed by >5% body weight loss, presence of diarrhea, and positive fecal occult blood tests. To ensure methodological consistency, all experimental manipulations, including gavage and sample collection, were conducted by the same trained researcher under identical conditions.

2.4. Preparation of Fecal Microbiota Suspension

Five healthy male C57BL/6 donor mice of similar weight were selected to prepare the fecal microbiota suspension. Fresh fecal pellets were collected in the early morning and processed within 30 min under aseptic conditions in a laminar flow hood to preserve microbial viability. Each gram of feces was homogenized in 10 mL of sterile saline

using a glass homogenizer and filtered through a 100-mesh stainless steel sieve to remove undigested residues. The filtrate was centrifuged at 1500 rpm for 5 min, and the supernatant was discarded. The resulting pellet was resuspended in sterile phosphate-buffered saline (PBS) and washed twice under the same conditions to eliminate impurities and non-bacterial debris. The final bacterial suspension (approximately 10^8 CFU/mL, determined by turbidimetric method) was obtained by resuspending the pellet in an equal volume of PBS. After Gram staining confirmed the absence of contamination, the suspension was stored at 4 ℃ in sterile, airtight tubes and used within 24 h to ensure microbial activity and compositional stability during transplantation.

2.5. Disease Activity Index (DAI) Assessment

Disease severity was evaluated daily using a modified scoring system described by Okayasu et al. [33], including three parameters: body weight loss, stool consistency, and fecal occult blood. The scoring criteria were as follows:

Body weight loss: 0 = none; 1 = 1–5%; 2 = 5–10%; 3 = 10–20%; 4 = >20%;

Stool consistency: 0 = normal; 2 = loose stool; 4 = diarrhea with gross bleeding;

Occult/gross blood: 0 = negative; 2 = occult positive; 4 = visible blood.

The total DAI score was calculated as:

DAI = (score for weight loss + stool consistency score + occult blood score)/3.

Two blinded observers independently evaluated all animals, and the average of both readings was used for analysis to minimize subjective bias.

2.6. Sample Collection

Twenty-four hours after the final FMT administration, mice were fasted overnight (12 h) but allowed free access to water. Mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.03 mL/10 g body weight), and blood was collected by enucleation of the eyeball. Samples were kept at 4 ℃ for 2 h, then centrifuged at 2000 rpm for 15 min. The supernatant serum was collected and stored at −80 ℃ until analysis.

Following euthanasia, the entire colon was excised from the cecum to the anus, measured for length, and visually inspected for congestion, edema, and erosion. Representative colon tissues were photographed for gross morphological comparison among groups.

2.7. ELISA Detection of Inflammatory Cytokines

Serum levels of TNF-α, IL-6, IL-1β, IL-4, and IL-10 were quantified using commercial ELISA kits following manufacturer instructions. Briefly, serum samples and standards were added to 96-well plates in duplicate. Plates were incubated at 37 ℃ for 60 min, washed five times, followed by incubation with enzyme-conjugated secondary antibodies for 30 min. After substrate reaction, absorbance was measured at 450 nm using a microplate reader (Bio-Rad, USA). Cytokine concentrations were determined based on standard curves. Each assay was repeated three times to ensure data reproducibility.

2.8. Statistical Analysis

All data were analyzed using SPSS 20.0 software (IBM, USA). Results are expressed as mean ± standard deviation (Mean ± SD). Differences among groups were assessed by one-way analysis of variance (ANOVA), followed by the least significant difference (LSD-*t*) test for pairwise comparisons. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Establishment of the DSS-Induced Ulcerative Colitis Model

After administration of 3% DSS for seven consecutive days, mice in both the model group (B) and FMT-treated group (C) exhibited typical clinical manifestations of ulcerative colitis, including reduced locomotor activity, dull fur, decreased appetite, loose stools, and positive fecal occult blood. In contrast, the control group (A) maintained normal activity, smooth fur, and formed stools. These results confirmed the successful establishment of the DSS-induced UC model.

3.2. Effect of FMT on Disease Activity Index (DAI)

As shown in **Figure 1**, DAI scores began to increase markedly from day 3 after DSS administration and peaked on day 7 in the model group, indicating progressive disease activity. DAI scores in the DSS model group were significantly higher than those in the control group ($p < 0.01$).

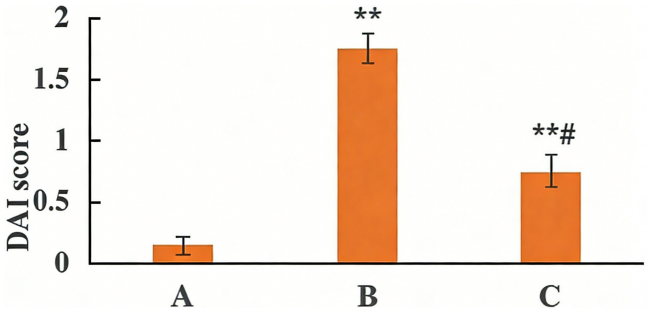

Figure 1. Changes in disease activity index (DAI) scores among the three groups of mice.
Note: ** indicates $p < 0.01$ vs. Group A; # indicates $p < 0.05$ vs. Group B.

After seven days of FMT treatment, DAI scores were significantly reduced compared with the DSS model group ($p < 0.01$), with an average reduction of approximately 35–40%. The decrease reflected improvement in stool consistency and a decline in the occurrence of visible bleeding. These findings indicate that FMT effectively attenuated DSS-induced clinical symptoms and ameliorated disease severity.

3.3. Effect of FMT on Colon Length and Macroscopic Morphology

As shown in **Figure 2**, colon shortening—a hallmark of colonic inflammation—was evident in the DSS-induced mice. The average colon length in the control group was 9.13 ± 0.42 cm, whereas DSS treatment significantly reduced colon length to 6.07 ± 0.35 cm ($p < 0.01$). FMT treatment partially reversed this reduction, with the average colon length restored to 7.54 ± 0.28 cm ($p < 0.05$ vs. DSS group). Qualitative histological observation suggested attenuation of mucosal injury and inflammatory features following FMT treatment. In the FMT-treated group, mucosal edema and ulceration were markedly alleviated, and the overall appearance approached normality.

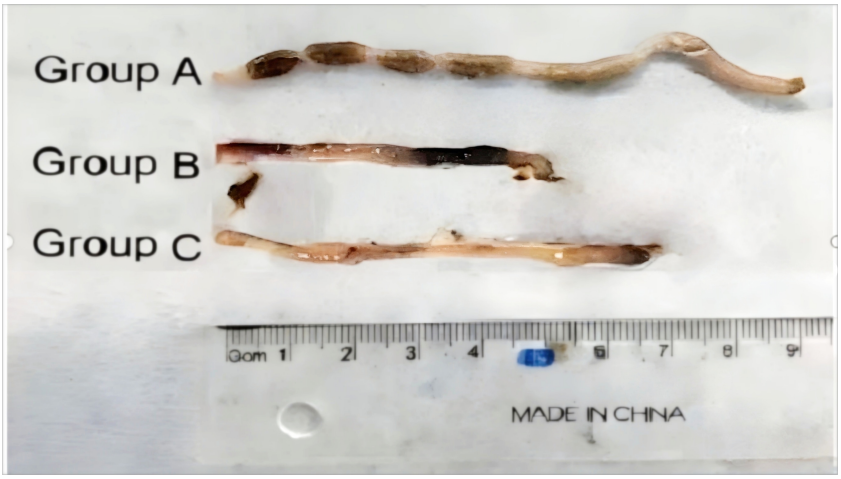

Figure 2. Representative macroscopic images of mouse colons in the different group.

3.4. Serum Levels of Inflammatory Cytokines

To assess systemic inflammation and immune regulation, serum concentrations of proinflammatory cytokines (TNF-α, IL-6, IL-1β) and anti-inflammatory cytokines (IL-4, IL-10) were measured using ELISA.

As summarized in **Table 1**, DSS exposure markedly increased serum levels of TNF-α, IL-6, and IL-1β ($p < 0.01$ vs. control), while significantly decreasing IL-4 and IL-10 levels ($p < 0.01$ vs. control), indicating a pronounced systemic inflammatory response and cytokine imbalance. FMT intervention reversed these alterations: levels of TNF-α, IL-6, and IL-1β were significantly reduced by approximately 9.1%, 27.4%, and 17.6%, respectively ($p < 0.01$ vs. DSS group), whereas IL-4 and IL-10 levels increased by 118% and 151%, respectively ($p < 0.01$ vs. DSS group).

Table 1. Serum Levels of Inflammatory Cytokines in thegroups (Mean ± SD).

Group	IL-6 (ng/mL)	IL-1β (pg/mL)	TNF-α (ng/mL)	IL-4 (pg/mL)	IL-10 (pg/mL)
A (Control)	13.52 ± 4.67	12.88 ± 7.40	14.74 ± 0.22	103.31 ± 9.86	174.26 ± 10.26
B (Model)	74.61 ± 5.64[a]	76.54 ± 6.52[a]	17.17 ± 0.23[a]	17.05 ± 10.33[a]	33.13 ± 11.13[a]
C (FMT)	54.21 ± 6.32[ab]	63.01 ± 7.12[ab]	15.61 ± 0.28[ab]	37.19 ± 7.56[ab]	50.25 ± 10.86[ab]
F value	999.372	720.807	438.542	593.744	1031.385
p value	0.000	0.000	0.000	0.000	0.000

Note: a: $p < 0.01$, vs. Group A; b: $p < 0.01$, vs. Group B.

These data demonstrate that FMT effectively downregulated proinflammatory cytokines and enhanced anti-inflammatory cytokines, thereby restoring the Th1/Th2 cytokine balance disrupted in DSS-induced colitis.

4. Discussion

Ulcerative colitis (UC) is a chronic, relapsing inflammatory bowel disease (IBD) defined by persistent mucosal inflammation, epithelial injury, and ulceration of the colonic mucosa, often extending beyond the gut to systemic immune dysregulation. Conventional therapies—ranging from aminosalicylates and corticosteroids to immunosuppressants and biologic agents such as anti-TNF-α antibodies—aim primarily at symptom control and acute inflammation. However, they come with significant limitations: systemic toxicities, declining efficacy, high relapse rates following cessation, and substantial cost burdens [11,20,34]. These limitations underscore the urgent need for novel therapeutic strategies that address the root causes of UC rather than merely suppressing downstream inflammatory sequelae.

In this context, fecal microbiota transplantation (FMT) has emerged as a compelling microbiome-based intervention. By restoring gut microbial homeostasis and thereby recalibrating host immune responses, FMT offers a fundamentally different therapeutic paradigm [21–24]. In the present study, we leveraged the dextran sulfate sodium (DSS)-induced UC mouse model [33]—which replicates key features of human UC, including weight loss, diarrhea, hematochezia, mucosal injury, and colon shortening—to explore the therapeutic efficacy and underlying immunomodulatory mechanisms of FMT.

Our results demonstrate that FMT significantly ameliorates UC phenotypes in treated mice: disease activity indices declined, colon lengths recovered, body weight stabilized, and mucosal architecture improved. Qualitative histological observations are consistent with the overall phenotypic improvement observed following FMT—findings that are consistent with previous reports in DSS-induced colitis models [27,35–38].

4.1. Immune Modulation via Pro- and Anti-Inflammatory Cytokine Regulation

One of the core revelations of our study lies in the bidirectional immunoregulatory capacity of FMT. Immune dysregulation in UC is commonly reflected by abnormal expression of pro- and anti-inflammatory cytokines. Th1 and Th17 cells facilitate pro-inflammatory cytokine release (TNF-α, IL-6, IL-1β), activate NF-κB and JAK/STAT signaling, and maintain chronic mucosal injury [26,37–40]. In contrast, Th2 and Treg populations secrete IL-4, IL-10, and other anti-inflammatory mediators to suppress macrophage activation, promote tissue repair, and restore immune homeostasis [41,42]. These observations are consistent with Tang [43], Wang et al. [44] and Morshedbak et al. [45], who reported similar immunological shifts following FMT. It is important to emphasize that the present study assessed serum cytokine concentrations and did not include direct immunophenotyping of T-cell subsets or innate immune populations. Therefore, the observed changes should be interpreted as cytokine-level immune modulation rather than definitive evidence of cellular immune reprogramming. Direct evaluation of immune-cell subsets (e.g., Th1, Th17, or Treg populations) would require additional markers such as IFN-γ, IL-17, and Foxp3, which were not assessed in this study.

Mechanistically, this immunomodulation appears to be achieved through interconnected pathways:

(1) Microbial-metabolite regulation: FMT restores the gut microbial ecosystem, normalizes production of SCFAs—especially butyrate, which acts as a histone deacetylase inhibitor (HDACi), up-regulates Foxp3 expression, promotes Treg differentiation, stimulates IL-10 and TGF-β secretion, and supports regulatory immune phenotypes [37,46]. SCFAs have been shown to influence immune regulation in multiple experimental systems. Although SCFAs and regulatory T-cell markers were not measured in the present study, the observed cytokine shifts are compatible with previously described microbiota-immune interactions.

(2) Signaling-pathway modulation: Following FMT, suppression of NF-κB and JAK/STAT activation reduces transcription of pro-inflammatory genes, while activation of PPARγ and Nrf2 pathways mitigates oxidative stress and protects epithelial cells from apoptosis [17,47,48]. However, these pathways were not directly examined in the present study, and any mechanistic linkage should be considered hypothetical and literature-based.

(3) Enhancement of mucosal barrier defence: FMT promotes renewal of the mucus layer, fosters goblet cell regeneration and mucin secretion, and reduces translocation of pathogen-associated molecular patterns (PAMPs) across the epithelium—thus curbing excessive innate immune activation [22]. Although we did not measure tight-junction proteins (e.g., ZO-1, Occludin), qualitative tissue morphology is compatible with reduced mucosal injury, although barrier function was not directly assessed. Collectively, FMT's effect constitutes a coordinated "microbiota–immune–inflammation" regulatory axis rather than a unidimensional anti-inflammatory action.

4.2. Gut Microbiota–Host Immune Crosstalk

The bidirectional relationship between gut microbiota and host immunity is central to UC pathophysiology. Dysbiosis—characterized by reduced microbial diversity, loss of beneficial taxa, and expansion of pathobionts—induces aberrant metabolite production, disrupts epithelial–immune cell communication, and triggers unchecked immune activation [13–18]. Mounting evidence indicates that the biological effects of FMT extend beyond simple microbial replenishment; through reconstructing microbial community structure and restoring metabolic functionality, FMT recalibrates immune responses at both mucosal and systemic levels [19].

Specific microbial taxa demonstrate targeted immunoregulatory roles: for example, *Clostridium butyricum* produces butyrate and salicylic acid derivatives with anti-inflammatory properties, and *Bacteroides fragilis* secretes polysaccharide A that promotes IL-10-producing Tregs [22, 49]. In our study, elevated IL-10 and IL-4 following FMT support the hypothesis that beneficial microbes encourage regulatory immune phenotypes. Moreover, current knowledge suggests that microbial restoration after FMT exerts downstream effects on antigen-presenting cells, including attenuation of dendritic-cell co-stimulatory molecule expression (CD80/CD86), normalization of pattern-recognition receptor signaling, and promotion of M2-type macrophage polarization [19,25,50,51]. These processes attenuate inflammation and support tissue repair. Thus, FMT functions as a potent immunemodulating intervention that transcends mere microbial reconstitution, providing a functional restoration of gut-immune crosstalk and interrupting the pathological positive-feedback cycle that sustains chronic colitis.

4.3. Comparative Advantage over Conventional Therapies

Conventional UC therapies, while effective in acute inflammation, insufficiently address the underlying dysbiosis and immune imbalance, and pose risks of side effects and relapse [11,20,34]. FMT offers a transformative therapeutic alternative: by targeting microbial dysregulation, modulating metabolism, and re-shaping immunological networks, it adopts a holistic systems-medicine approach.

Advantages include:

(1) Etiology-focused intervention: FMT addresses gut-microbiota imbalance—the putative root cause of UC—rather than symptomatic suppression.

(2) Multi-system regulation: FMT simultaneously modulates metabolic, immune and epithelial repair pathways, achieving integrated therapeutic effects.

(3) Biologically natural origin: FMT leverages live microbial communities derived from healthy donors, thus reducing systemic toxicity and likelihood of drug-resistance.

Clinical evidence underscores these advantages: Borody et al. [30] reported complete remission in 67.7% of

UC patients post-FMT, with partial remission in 24.2%. Paramsothy et al. [31] demonstrated ~60% clinical remission within eight weeks in a randomized multi-donor trial. Chinese studies show that FMT combined with conventional therapy significantly reduces TNF-α and IL-6 levels [32]. Our findings extend this clinical evidence by demonstrating that FMT reliably reshaped cytokine signatures and restored immune balance, supporting the mechanistic plausibility of clinical benefit.

Innovation and translational implications:

This work offers several significant innovations:

(1) **Bidirectional immune regulation**: FMT enhances anti-inflammatory mediators while simultaneously suppressing pro-inflammatory factors, restoring immune homeostasis.
(2) **Integrated analytical framework**: Although our study centered on cytokine regulation, the combined phenotypic readouts (DAI, colon morphology, and cytokine profiles) provide a coherent mechanistic link that connects microbial restoration to immune regulation and tissue repair, culminating in improved mucosal immunity.
(3) **Standardized donor usage**: Utilizing consistent donor microbiota reduces variability and bolsters reproducibility, enhancing translational relevance.

Additionally, this study responds directly to ongoing debates about FMT mechanistic specificity by demonstrating that immune effects (e.g., IL-10 elevation, TNF-α suppression) occur even in the absence of sequencing or pathway assays. This suggests that cytokine rebalancing represents an early and measurable readout of FMT efficacy, offering a practical biomarker for future translational studies.

These innovations bear substantial translational import. The present results establish a foundational dataset for developing precision FMT protocols or core microbial consortia, advancing gut-immune axis-targeted therapies for UC and potentially other immune-mediated inflammatory diseases.

4.4. Study Limitations and Future Directions

Notwithstanding its contributions, the study has limitations. First, cytokine assessments were limited to serum; we did not interrogate tissue-resident immune cell infiltration or signaling protein expression. Second, microbial composition and metabolite (e.g., SCFAs, bile acid derivatives) dynamics following FMT were not profiled, limiting construction of a full "microbiota–metabolite–immune" causal chain. Third, FMT parameters such as dosing, donor selection, and treatment duration remain unresolved and were not standardized in this study. Fourth, our cytokine panel did not include key Th1/Th17/Treg markers such as IFN-γ, IL-17, or Foxp3. Inclusion of these markers in future studies would permit more comprehensive mapping of FMT-associated immune reprogramming. In addition, histological evaluation of colonic tissue in this study was limited to qualitative morphological observation, and standardized histological scoring systems (e.g., inflammation severity or crypt damage scores) were not applied. This limitation constrains the quantitative interpretation of tissue-level inflammatory improvement following FMT. Accordingly, histological findings in the present study are intended to serve as supportive descriptive evidence rather than definitive quantitative endpoints.

Future studies should focus on:

(1) Multi-omics integration: Deploying 16S rRNA sequencing, metabolomics, transcriptomics and proteomics to delineate key regulatory pathways of FMT.
(2) Signal network validation: Systematically investigating NF-κB, STAT3, Nrf2, TGF-β-Smad and related pathways to map mechanistic cascades.
(3) Personalised donor and synthetic consortia strategies: Designing defined microbial communities ("core taxa") and adopting precision donor-recipient matching to optimize therapeutic outcomes.
(4) Clinical translation and longitudinal safety assessment: Conducting comprehensive long-term studies evaluating gut homeostasis, immune function, metabolism and safety of FMT in human UC cohorts.
(5) Expanded immune-phenotyping using flow cytometry or single-cell sequencing to quantify Th1/Th2/Th17/Treg subsets and delineate cell-type-specific responses to FMT.
(6) Quantitative histopathological assessment: Incorporating standardized histological scoring systems in fu-

ture studies to strengthen tissue-level evaluation of FMT efficacy and to complement cytokine-based immune readouts.

5. Conclusion

In conclusion, this study provides evidence that fecal microbiota transplantation (FMT) provides notable therapeutic and immunomodulatory benefits in dextran sulfate sodium (DSS)-induced ulcerative colitis (UC) in mice. FMT attenuated disease activity, improved macroscopic colonic features, and was associated with reduced mucosal injury. These phenotypic improvements were accompaniedby a shift in systemic cytokine profiles, characterized by reduced levels of TNF-α, IL-6, and IL-1β and elevated IL-4 and IL-10, suggesting partial restoration of pro- and anti-inflammatory balance disrupted during colitis.

The immunological improvements observed in this study are consistent with a coordinated immunomodulatory influence of FMT, as inferred from prior microbiota–immune research. Although these mechanisms are widely supported by existing literature, the present study did not directly evaluate microbial composition, short-chain fatty acid production, tight junction protein expression, or intracellular signaling cascades; therefore, mechanistic interpretations should be considered preliminary. Likewise, key markers of Th1, Th17, and Treg activity—such as IFN-γ, IL-17, and Foxp3—were not assessed, limiting the ability to characterize the broader T-cell regulatory landscape influenced by FMT.

Nevertheless, the cytokine alterations documented here support the role of FMT as a promising microbiota-based immunomodulatory strategy for UC. The findings underscore the importance of targeting gut-immune crosstalk as a therapeutic principle and highlight the need for more detailed mechanistic studies. Future research incorporating multi-omics profiling, including microbiome, metabolome, and immunome analyses, together with comprehensive immune-cell phenotyping and pathway-level validation, will be essential to elucidate the causal links between microbial reconstruction, immune reprogramming, and mucosal healing. Such advances will help refine donor selection, optimize FMT protocols, and support the development of standardized and potentially personalized microbiota-based interventions for restoring immune equilibrium and intestinal homeostasis in UC and related immune-mediated inflammatory diseases.

Author Contributions

Experimental procedures, Y.X. and D.C.; data analysis, J.Z. and A.Z.; writing, L.L.; project management, L.L.; manuscript review, X.B. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement

All experimental procedures were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals issued by the Ministry of Science and Technology of China and were approved by the Biological and Medical Ethics Committee of Pingdingshan University (2023-008).

Informed Consent Statement

Not applicable.

Data Availability Statement

The authors support the journal's commitment to data sharing for research transparency. However, the raw data of this study (including animal experimental records, serum cytokine detection results, and colonic morphological observation data) cannot be publicly shared due to ethical constraints and compliance with the Guidelines for the Care and Use of Laboratory Animals. These data are protected by the ethical approval of Pingdingshan Univer-

sity (Approval No.: 2023-008). Qualified researchers may request de-identified data for non-commercial academic purposes by contacting the corresponding author (Xianguang Bai, E-mail: 3562@pdsu.edu.cn) with a detailed research proposal and compliance commitment.

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Conflicts of Interest

The authors declare no conflict of interest.

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