

Original Research

The Protective Role of TLR4 in Intestinal Epithelial Cells through the Regulation of the Gut Microbiota in DSS-Induced Colitis in Mice

Yun-Jie Shi^{1,2,†}, Cheng Huang^{1,†}, Pi-Qing Gong², Cong Liu^{3,*}, Zhi-Qian Hu^{2,4,*},
Hao Wang^{1,*}¹Department of Anorectal Surgery, Changhai Hospital, Naval Medical University (Second Military Medical University), 200433 Shanghai, China²Department of Anorectal Surgery, Changzheng Hospital, Naval Medical University (Second Military Medical University), 200003 Shanghai, China³Department of Radiation Medicine, Faculty of Naval Medicine, Naval Medical University (Second Military Medical University), 200433 Shanghai, China⁴Department of General Surgery, Tongji Hospital, School of Medicine, Tongji University, 200065 Shanghai, China*Correspondence: victorliu20102020@163.com (Cong Liu); huzhiq163@163.com (Zhi-Qian Hu); wanghaohh@vip.126.com (Hao Wang)

†These authors contributed equally.

Academic Editor: Ru Chen

Submitted: 7 January 2023 Revised: 18 March 2023 Accepted: 22 March 2023 Published: 23 August 2023

Abstract

Background: The cause of ulcerative colitis (UC) is not yet fully understood. Previous research has pointed towards a potential role for mutations in nucleotide-binding oligomerization domain-containing protein 2 (NOD2) in promoting the onset and progression of inflammatory bowel disease (IBD) by altering the microbiota of the gut. However, the relationship between toll-like receptor 4 (TLR4) and gut microbiota in IBD is not well understood. To shed light on this, the interaction between TLR4 and gut microbiota was studied using a mouse model of IBD. **Methods:** To examine the function of TLR4 signaling in intestinal injury repair, researchers developed Dextran Sulfate Sodium Salt (DSS)-induced colitis and injury models in both wild-type (WT) mice and TLR4 knockout (TLR4-KO) mice. To assess changes in the gut microbiota, 16S rRNA sequencing was conducted on fecal samples from both the TLR4-KO and WT enteritis mouse models. **Results:** The data obtained depicted a protective function of TLR4 against DSS-induced colitis. The gut microbiota composition was found to vary considerably between the WT and TLR4-KO mice groups as indicated by β -diversity analysis and operational taxonomic units (OTUs) cluster. Statistical analysis of microbial multivariate variables depicted an elevated abundance of *Escherichia coli/Shigella*, *Gammaproteobacteria*, *Tenerlucetes*, *Deferribacteres*, *Enterobacteria*, *Rikenellaceae*, and *Proteobacteria* in the gut microbiota of TLR4-KO mice, whereas there was a considerable reduction in Bacteroidetes at five different levels of the phylogenetic hierarchy including phylum, class, order, family, and genus in comparison with the WT control. **Conclusions:** TLR4 may protect intestinal epithelial cells from damage in response to DSS-induced injury by controlling the microbiota in the gut.

Keywords: 16S rRNA sequencing; toll-like receptor 4; gut microbiota; DSS-induced colitis

1. Introduction

A chronic inflammatory disorder of the colon, ulcerative colitis (UC) [1], together with Crohn's disease (CD), constitutes two primary types of inflammatory bowel disease (IBD) [2]. The etiology of IBD is still elusive and is supposed to be associated with environmental stimuli, immunological factors, and genetic susceptibilities [3]. Studies have demonstrated that immunodeficiency is a major cause of inflammation and tissue damage [4]. Excessive activation of the toll-like receptor (TLR)/nuclear factor κ B (NF- κ B) signaling pathway has been reported to promote the development of UC [5,6], but few studies have reported its protective effect on the intestinal epithelium. Our previous findings indicated the repairing effect of TLR4 on intestinal damage induced by DSS and up-regulated IL6, CCL2, and CSF3 [7].

As a vital part of the body, gut microbiota in the digestive tract contains large numbers of microorganisms [8]. Development in the body of the mother and the breastfeed-

ing pathways after birth shape the gut microbiota of the infant, which gradually matures with environmental exposure during childhood. The gut microbiota from late childhood to adulthood remains quite stable, but as the immune status declines, the diversity of gut microbiota will decrease in the elderly [9]. The core functions of mature healthy gut microbiota include the short-chain fatty acids (SCFA) production through complex polysaccharides fermentation and the formation of certain lipopolysaccharides (LPS), as well as the biosynthesis of some essential amino acids and vitamins [10]. Gut microbiota dysfunction may cause damage to the intestinal mucosal immune barrier, which is believed to be strongly associated with the onset and progression of UC [11]. Microbiota of the gut has been reported as vital concerning inflammation in IBD in various experiments [12], which may help better understand the interaction between the microbiota in IBD and the immune system. TLRs and the cytoplasmic receptor, known as the nucleotide-binding oligomerization domain-containing



protein 2 (NOD2) [13], are sensors for pathogen-associated molecular patterns (PAMPs) that include peptidoglycan, lipoteichoic acid, endotoxin, flagellin, lipopolysaccharide (LPS) and muramyl-peptide (MDP) [14–16]. NOD2 mutations have been reported to have a significant impact on the gut microbiota composition by increasing the number of mucosa-adherent bacteria [17] and decreasing the anti-inflammatory cytokine interleukin 10 (IL-10) transcription [18], thereby promoting the development of IBD. However, the relationship between TLR4 and gut microbiota in IBD remains poorly understood.

A variety of chemically-induced colitis models have been most utilized on the laboratory scale [19], among which the DSS-induced mouse colitis model is the predominantly utilized IBD animal model. DSS is formed by the esterification of dextran and chlorosulfonic acid, with the molecular formula of $(C_6H_7Na_3O_{14}S_3)_n$ [20]. The model uses a chemically formed DSS with anticoagulant properties to induce intestinal inflammation in mice by inducing epithelial damage. This animal model is suitable for the study of IBD owing to simple preparation, short molding time, and good reproducibility [21]. This research focused on exploring the function of TLR4 in DSS-induced colitis in wild-type (WT) and TLR4 knockout (TLR4-KO) mouse models and analyzing the differences (variations) in the microbiota of the gut between the two models using 16S rRNA sequencing.

2. Materials and Methods

2.1 Animals

Animals used in this study included C57BL/6 WT adult male mice weighing 25–30 g, and C57BL/6 TLR4 gene-deleted type TLR4^{-/-} mice [22] weighing 24–33 g (Model Animal Research Center of Nanjing University, Nanjing, China). The TLR4^{-/-} mice were fertile with no developmental abnormalities. All the mice were raised in specific pathogen-free (SPF) conditions in the Laboratory Animal Center of the Naval Medical University (Shanghai, China). Approval for the research was granted by the Ethics Committee of the Naval Medical University per the Declaration of Helsinki.

2.2 DSS-Induced Colitis

The DSS-induced colitis model in all WT and TLR4^{-/-} mice was constructed by dividing them into WT and TLR4^{-/-} groups with 12 mice in each group to observe their survival time. For a period of seven days, the mice were administered 1.5% DSS [23] (40,000 kDa; ICN Biochemicals, Anaheim, California, USA) in their drinking water before switching to ordinary water. After the seven-day DSS treatment, the survival status of the mice in the two groups was observed daily. Six mice in each group received 1.5% DSS orally for seven days and were used to observe the general biological indicators including daily weight change and food and water intake (recorded on day

five) of the two groups. Six mice in each group receiving 1.5% DSS orally for five days were used for colon bleeding observation, peripheral blood analysis, and histopathological examination. Six mice in each group receiving 1.5% DSS orally for seven days were used to examine the fecal samples by means of 16S rRNA analysis.

2.3 Peripheral Blood Analysis

On day five after DSS treatment, about 2 mm of the mouse tail was cut off, from which about 40 μ L blood was sampled, and then transferred to a K2-EDTA containing Eppendorf (EP) tube, followed by repeated inversions of the tube. Blood cells were counted utilizing a small animal blood cell counter.

2.4 Intestinal Specimens and Histopathology

The mice were euthanized via cervical dislocation on the third or fifth day following DSS treatment, and the intestinal tissues were isolated for colon bleeding observation. The dehydration of the gut tissues was conducted in an ascending series of ethanol, cleared, paraffin-embedded, and serially sliced into 2–7 micron-thick sections utilizing a rotatory microtome (Aihua, Tianjin, China). Hematoxylin-eosin (H & E) staining was commonly used to stain the deparaffinized sections [24].

2.5 Collection of Mouse Fecal Samples

Six WT mice and six TLR4-KO mice were classified into two groups and orally administered with 1.5% DSS. Following DSS treatment, the mice in both groups were separately transferred to 12 clean cages lined with sterile filter paper on the seventh day. Immediately following defecation, fecal samples were placed in sterile centrifuge tubes and kept at -80°C for later examination and transplantation [25].

2.6 16S rRNA Analysis of Fecal Samples

Extraction of total genomic DNA through the DNA Extraction Kit (Tiangen Biotechnology Company, Beijing, China) was conducted following the instructions of the kit. DNA was quantified and its quality was examined through NanoDrop and agarose gel, respectively. Dilution of the extracted DNA to 1 ng/ μ L concentration was done and was subsequently kept at -20°C until further use. Barcoded primers and Takara Ex Taq (Takara, Tokyo, Japan) were used in PCR to amplify the bacterial 16S rRNA genes using the diluted DNA as a template. Concerning the amplification of the 16S rRNA genes' variable regions V3–V4, the universal primers 343 F and 798 R were employed. The primers were connected with an Illumina sequencing adapter with the reverse primer containing a sample barcode. PCR products were purified and the concentrations were adjusted for sequencing on an Illumina Miseq PE300 system (Oebiotech, Shanghai, China). Utilizing their distinctive barcodes, the samples' raw sequencing reads were

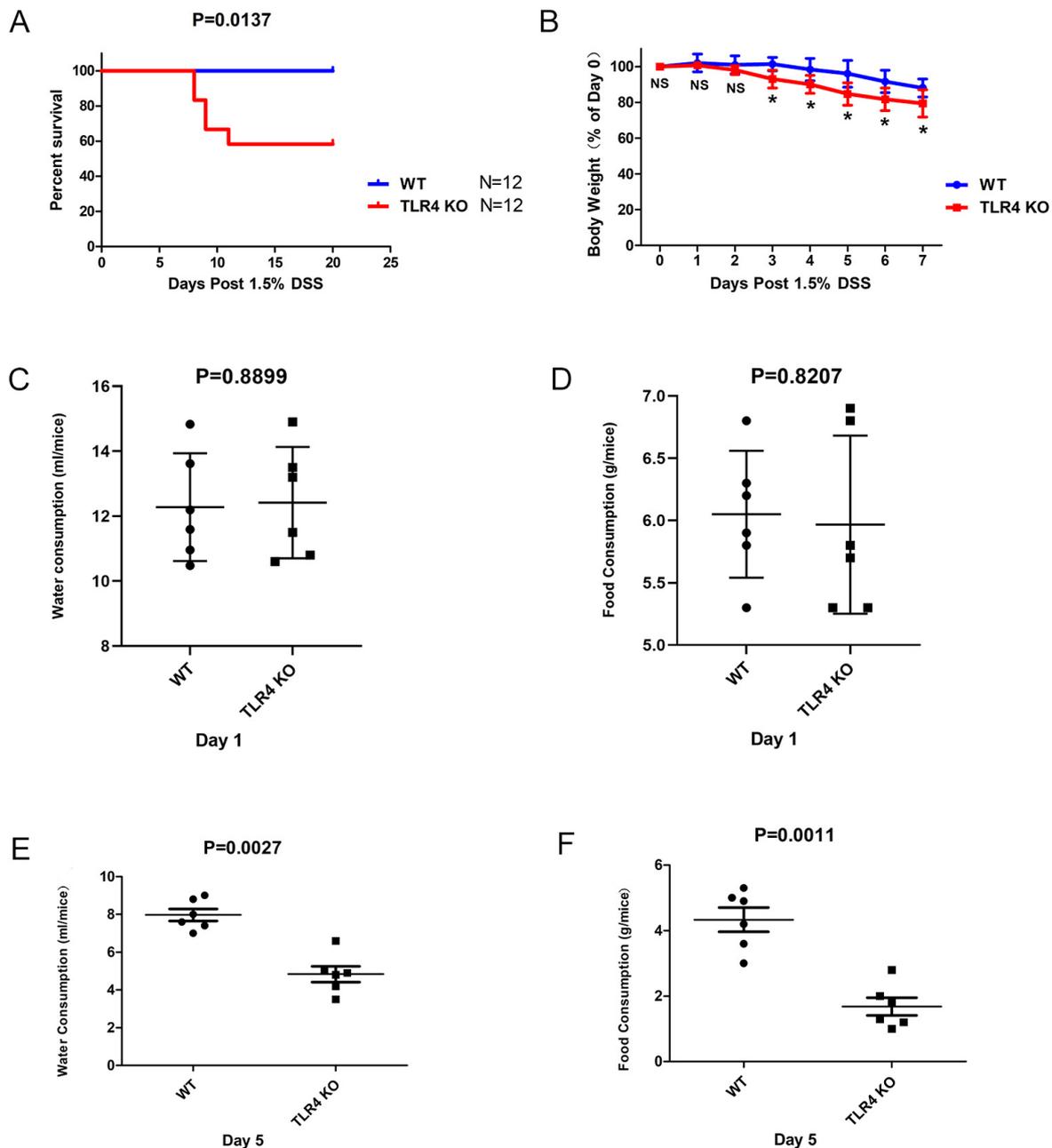


Fig. 1. Severe fatality and morbidity in TLR4-KO mice subsequent to orally administered 1.5% DSS. (A) WT and TLR4-KO mice were orally administered with 1.5% DSS for seven days. The survival duration was documented ($p = 0.0137$). (B) Body weight change (%) = (weight at day X / weight at day 0) \times 100%. (C) Water consumption (mL/mouse) and (D) food weight (g/mouse) of WT and TLR4-KO mice were recorded on day one ($p = 0.8899$, $p = 0.8207$). (E) Water consumption (mL/mouse) and (F) food weight (g/mouse) of WT and TLR4-KO mice were recorded on day five ($p = 0.0011$, $p = 0.0027$). * $p < 0.05$, denotes remarkable variation from the WT control group; NS, No significant difference detected. Kaplan–Meier plus Cox Regression Analysis was used in (A). Student’s *t*-test analysis was used in (B–F). Abbreviations: DSS, Dextran Sulfate Sodium Salt; WT, wild-type; TLR4-KO, TLR4 knockout.

sorted. After removing the barcode and linker, they were subjected to primer sequencing and clustering to generate operational taxonomic units (OTUs) using the software V search with a 97% similarity threshold [26]. All sequences were classified through the NCBI BLAST and SILVA databases [27]. R software (R-4.2.2, Auckland, New Zealand) was employed to carry out the 16S rRNA

analysis of the fecal samples. The raw data of 16S rRNA analysis obtained in this study are available in the Gene Expression Omnibus (GEO) database with accession number GSE227565.

Statistical treatment. Comparisons between TLR4^{-/-} and relevant control WT mice groups with DSS treatment were conducted by means of the Student’s

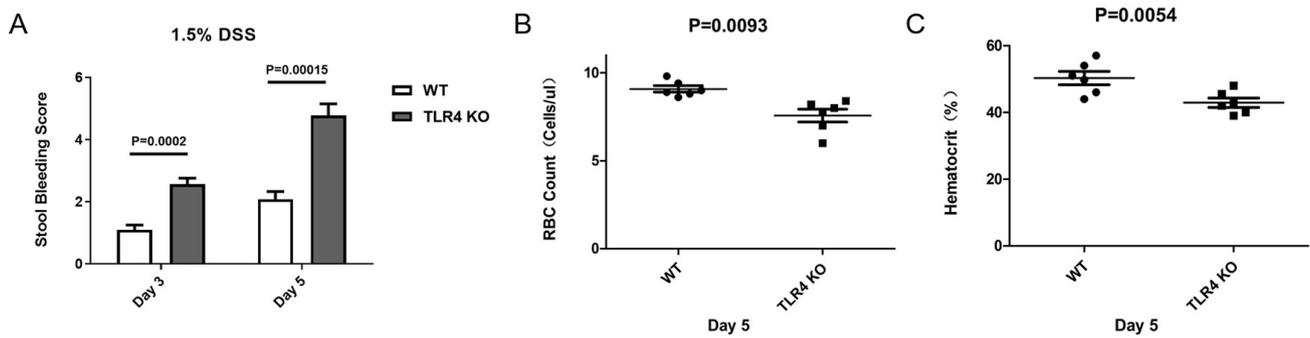


Fig. 2. Elevated susceptibility of TLR4-KO mice to contract anemia. (A) Stool bleeding Score on days three and five. The following is the stool bleeding score: 0 = absence of any gross blood visible in the entirety of the colon; 1 = presence of gross blood in <1/3 of the colon; 2 = 2/3; 3 => 2/3 of the colon. (B) RBC count in peripheral blood of the mice on day five subsequent to orally administered 1.5% DSS ($p = 0.0093$). (C) The hematocrit concentration in the peripheral blood of the mice on day five of orally administered 1.5% DSS ($p = 0.0054$). $p < 0.05$, denoting remarkable variation from the WT control group. Student's t -test analysis was utilized in this section. Abbreviation: RBC, red blood cell.

t -test analysis of variation. Survival differences between $TKR4^{-/-}$ and WT mice groups with DSS treatment were evaluated utilizing Kaplan–Meier plus Cox Regression Analysis. Statistical significance was associated with a p value < 0.05 . The QIIME package was utilized for the selection of the representative reading of each OTU. Estimator calculation (α -diversity and β -diversity) was conducted by the MOTHUR program [28]. Adonis and Anosim were used to assess the presence of any remarkable variation in the WT and TLR4 KO groups. ANOVA and LefSe methods were utilized to examine the species with considerable biological duplication variation in the statistical group. ANOVA was employed to examine OTU and species with remarkable variations at the phyla, class, order, family, genus, and species. LefSe analysis reveals the composition of different species in two or more groups of biological communities. Whereas the species were assessed through LefSe analysis for remarkable variation at the phyla, class, order, family, genus levels.

3. Results

3.1 TLR4-KO Mice were Severely Susceptible to DSS-Induced Colitis

First, the function of TLR4 in DSS-induced intestinal injury was explored. It was found that the mortality in TLR4-KO mice was notably elevated in contrast to WT mice ($p = 0.0137$) (Fig. 1A). There were remarkable variations in the dynamic change of body weight between the two groups on day seven. TLR4-KO mice showed considerably more body weight loss than WT controls subsequent to orally administered 1.5% DSS (Fig. 1B). Simultaneously, the protective function of TLR4 against DSS-induced acute intestinal damage was analyzed using some basic daily behavioral parameters. The water and food consumption of WT and TLR4-KO mice treated with 1.5% DSS-supplemented water was recorded for seven consec-

utive days per mouse. The result showed that no significant difference in the water-drinking ($p = 0.8899$) and food-ingestion ($p = 0.8207$) abilities between TLR4-KO and WT mice at day 1 (Fig. 1C,D). However, the food-ingestion ($p = 0.0011$) and water-drinking ($p = 0.0027$) abilities of TLR4-KO mice began decreasing significantly from day five in comparison with those of the WT mice (Fig. 1E,F).

3.2 TLR4-KO Mice Showed More Obvious Symptoms of Anemia and Bleeding in the Colon

Morphological changes of the mice in the two groups were determined after oral administration of 1.5% DSS. Intestinal bleeding was detected in TLR4-KO and WT mice on days three ($p = 0.0002$) and five ($p = 0.00015$) respectively, but the severity of symptoms was increased in the TLR4-KO group in comparison to the WT group (Figs. 2A,3A). Consistent with colon bleeding, TLR4-KO mice showed more severe anemia by quantifying the peripheral red blood cell count on day five (RBC Count: $p = 0.0093$; Hematocrit: $p = 0.0054$) in both groups (Fig. 2B,C). Simultaneously, intestinal pathologic evaluation was performed on day five after oral administration of 1.5% DSS. It was found that intestinal inflammation and tissue necrosis in TLR4-KO mice were more severe than those in WT mice (Fig. 3B). All the above results demonstrated that TLR4-KO elevated the severity of intestinal epithelial injury in DSS-induced enteritis in mice.

3.3 Comparisons of Differential Profiles of Gut Microbiota between TLR4-KO and WT Mice After DSS Treatment

Differences in the composition of the microbiota of the gut in fecal samples were compared through sequencing 16S rRNA gene between TLR4-KO and WT mice at day seven after orally administered 1.5% DSS using Operational Taxonomic Unit (OTU) [29] partitioning with 97% sequence similarity in gut microbiota. From the flower plot, shared intestinal microbiota OTUs of all fecal samples were

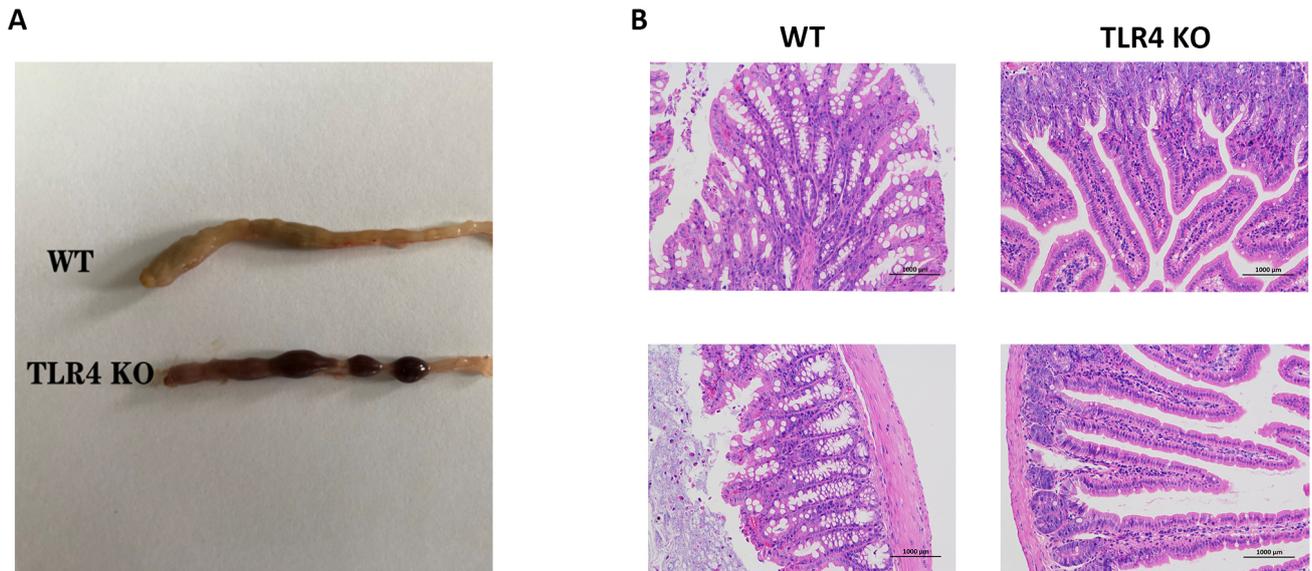


Fig. 3. Colon histopathology of the two mouse groups. (A) Photographs of bleeding colon from WT and TLR4-KO mice on day seven of orally administered 1.5% DSS. (B) H&E staining photographs of the colon in the two mice groups seven days following 1.5% DSS-infused drinking water treatment.

found to be 42, the number on the petal indicated the total OTUs of each sample minus the number of common OTUs (**Supplementary Fig. 1**). The abundance of each OTU was analyzed and the first 50 with the most abundant OTU were selected, an evolutionary tree was established, and the abundance of OTU in different samples was displayed with a heatmap graph (**Supplementary Fig. 2**). α -diversity [30] is defined as the microbiota diversity within a community. The diversity of microbiota has two aspects: one is the type of bacteria, and the other is the bacteria abundance uniformity. No significant difference in α -diversity value in the intestinal microbiota was observed between TLR4-KO and WT mice (**Supplementary Fig. 3A–C**). β -diversity [31] refers to the diversity of microbiota between different communities. β -diversity includes principal component analysis (PCA), principal coordinates analysis (PCoA), and Unweighted Pair Group Method with Arithmetic mean (UPGMA) analysis. PCA and PCoA present visual coordinates for the similarity or variation of data. These results demonstrated that the dots of the six WT and six TLR4 KO samples were close to the respective dots in the same group, while the dots in the TLR4-KO group were further away from those in the WT group (Fig. 4A,B). UPGMA revealed similarities between the samples or groups through the distance matrix algorithm. The circular tree was established by UPGMA analysis, showing that the gut microbiota composition varied considerably in the two groups (Fig. 4C). Hence, the intestinal microbiota composition in β -diversity varied considerably between TLR4-KO and WT mice after 1.5% DSS treatment.

3.4 Differences in the Change of Gut Microbiota Community between TLR4-KO and WT Mice after DSS Treatment

According to the microbiota taxonomic levels of phylum, class, order, family, and genus, the relative abundance of each sample at various levels was recorded. The average number of reads per sample was listed in **Supplementary Table 1**. After a seven-day treatment with 1.5% DSS, the dominant phylum, class, order, family, and genus in WT mice were *Bacteroidetes*, however, they were considerably reduced in TLR4-KO mice. Simultaneously, the phylum *Proteobacteria*, the class *Gammaproteobacteria* (Fig. 5A,B), the order *Enterobacteriales*, the family *Enterobacteriaceae*, and the genus *Escherichia/Shigella* and *Rikenellaceae* were increased in TLR4-KO mice in contrast to WT controls (**Supplementary Fig. 4A–C**). Furthermore, ANOVA statistical analysis was carried out on OTUs and the five aforementioned phylogenetic levels, and the first ten different OTUs (species abundances) were selected for relative abundance boxplot analysis for the sake of quickly obtaining intra- and inter-group differences between the dominant and differential species. The results of ANOVA difference statistics analysis showed at the family level abundant *Enterobacteriaceae*, *Moraxellaceae*, *Deferribacteraceae*, *Desulfovibrionaceae*, *Rikenellaceae*, *Xanthomonadaceae*, and *Pseudomonadaceae* in gut microbiota in TLR4-KO mice, and abundant *Bacteroidales*, *Pepococcaceae*, *Alcaligenaceae* and *Porphyromonadaceae* in gut microbiota in WT mice following DSS treatment (Fig. 6A,B). The linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe) analysis indicated that the gut microbiota genera were differentially

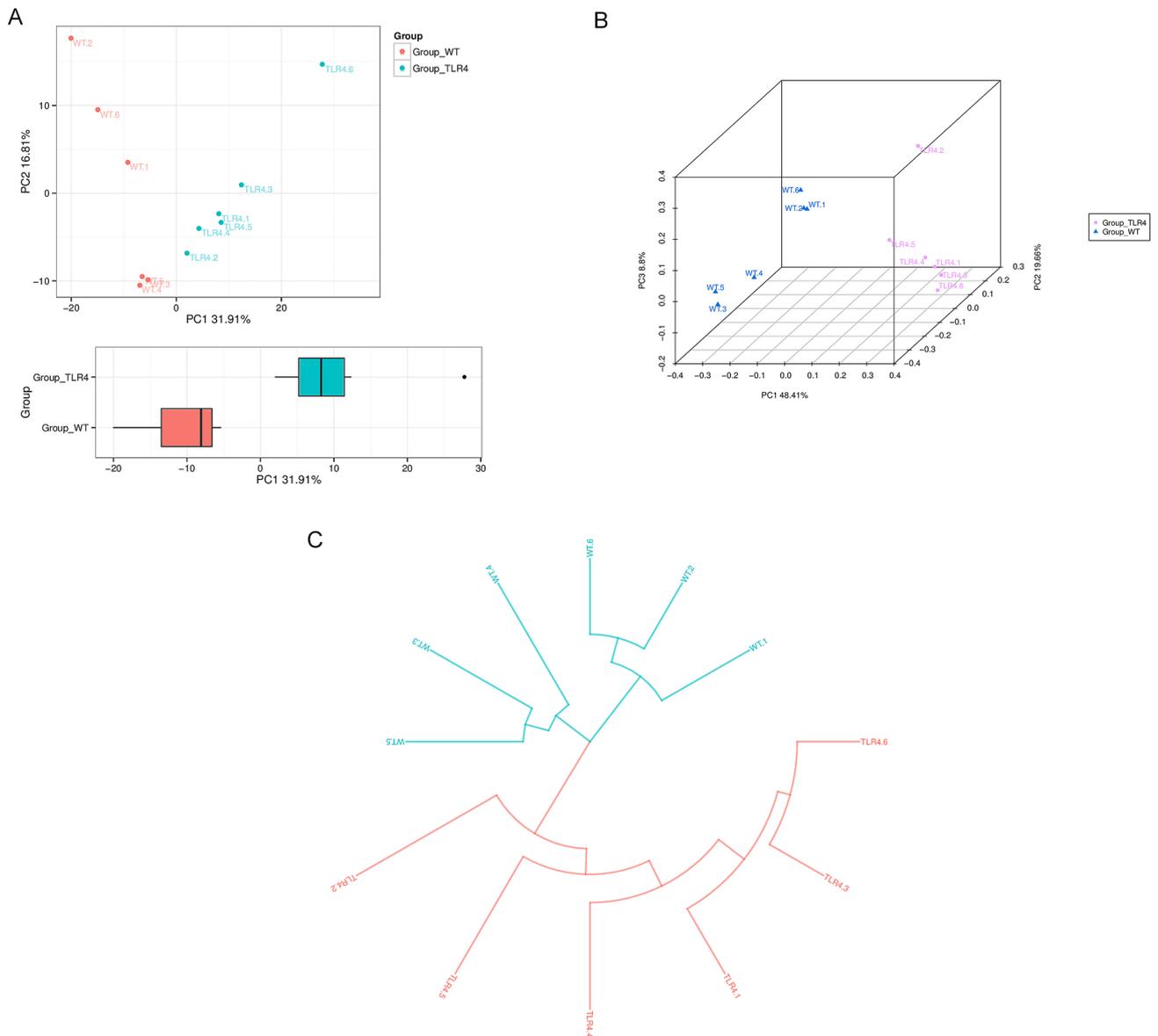


Fig. 4. β -diversity analysis on intestinal microbiota of WT and TLR4-KO mice. (A) Principal component analysis (PCA) analysis of gut bacteria data. (B) Gut bacteria principal coordinates analysis (PCoA) analysis. (C) The circular tree by Unweighted Pair Group Method with Arithmetic mean (UPGMA) analysis.

numerous between WT and TLR4-KO mice after orally administered 1.5% DSS. Gladography revealed a considerably increased abundance of *Bacteroidetes* at the genus level in WT mice, whereas an increased abundance of *Deferribacteres*, *Tenerlcutes*, and *Proteobacteria* was determined in TLR4-KO mice (Fig. 7).

4. Discussion

Due to the limitations of human differences, as well as ethical and moral factors, the pathogenesis of IBD has not been clearly defined, and there is no effective clinical treatment [32]. Therefore, it is very important to create a simple and easy animal model mimicking the clinical symptoms. The mature IBD models include spontaneous animal colitis

models, cell transplantation models, and chemical-induced models [33]. In this study, the spontaneous mouse colitis model induced by DSS was employed. DSS is a synthetic heparin polysaccharide and the DSS modeling method is simple, with good repeatability and maintenance. As the signs and pathological features of the model mimic human IBD, it is currently regarded as the gold standard in the field of colitis modeling research [34]. In this experiment, the mouse colitis model was induced by drinking DSS. After modeling, the weight of the mice decreased, and the intestinal histopathology showed inflammatory changes, confirming that the DSS-induced colitis mouse model was successfully established. In this study, survival, body weight change, basic daily behavioral parameters, and intestinal

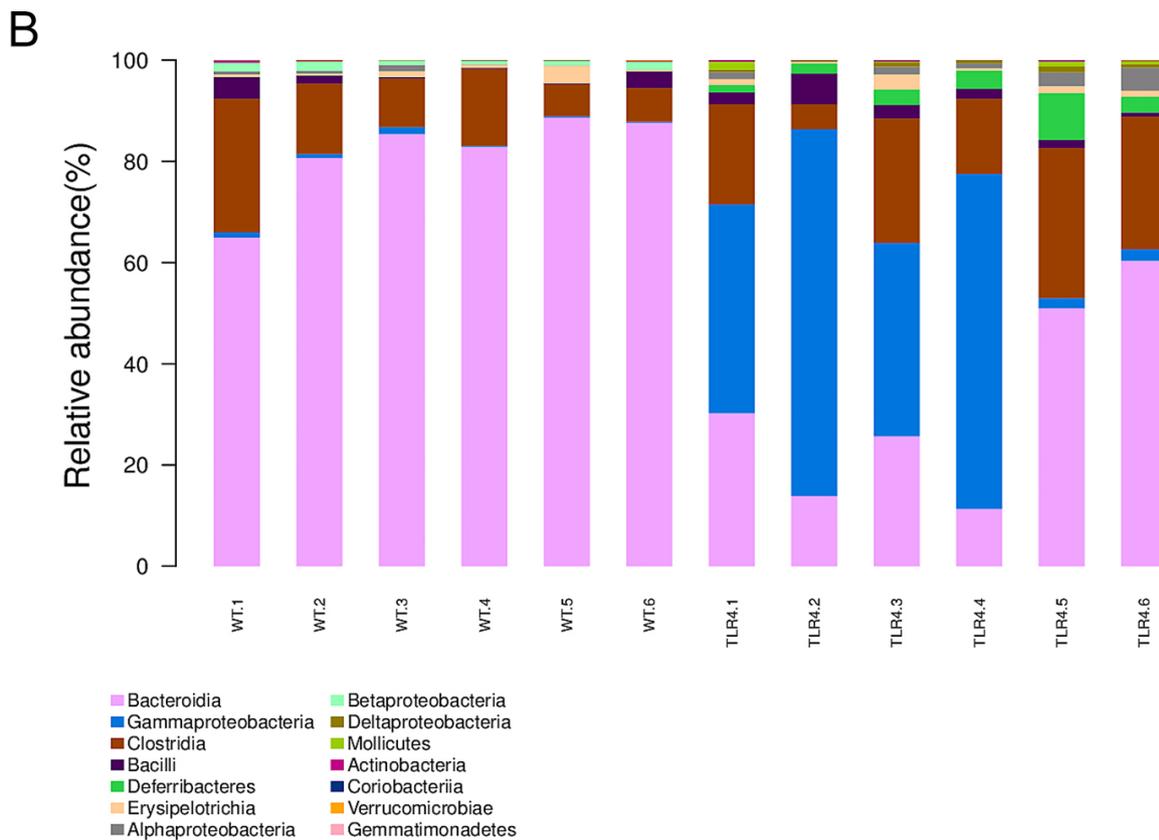
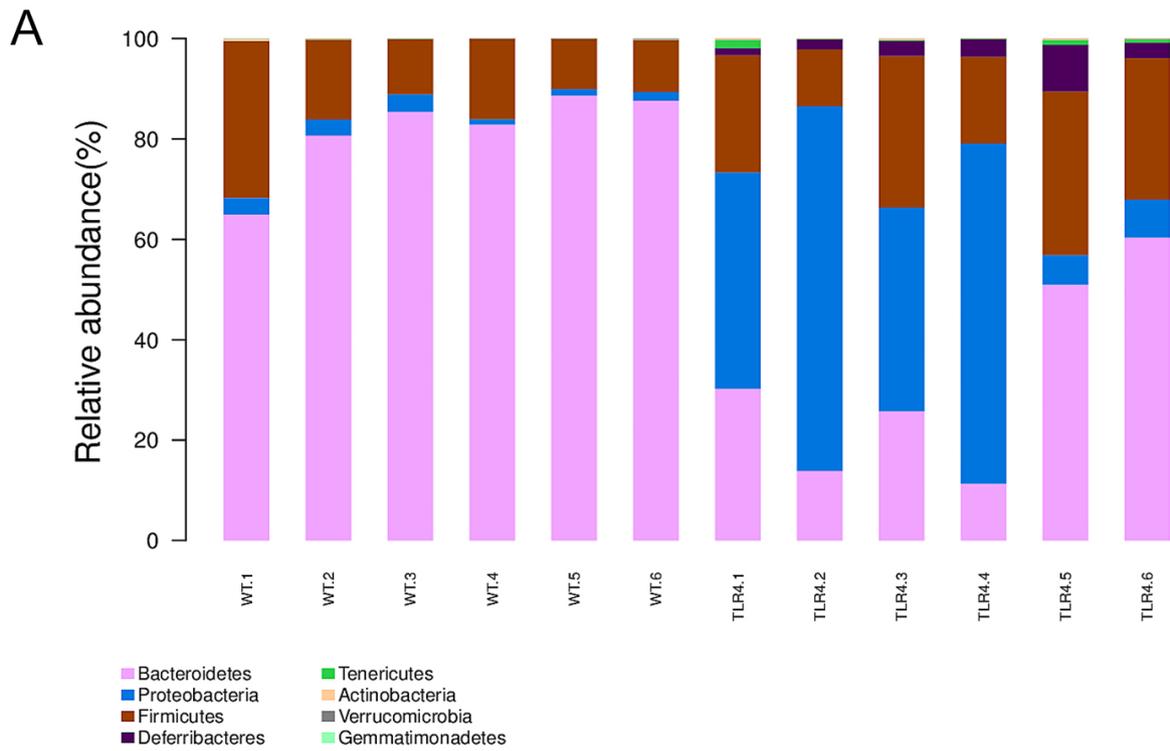


Fig. 5. Gut microbiota variation in TLR4-KO and WT mice after DSS treatment at levels of phylum and class. (A) The relative abundance of the main phylum. (B) The relative abundance of the main class.

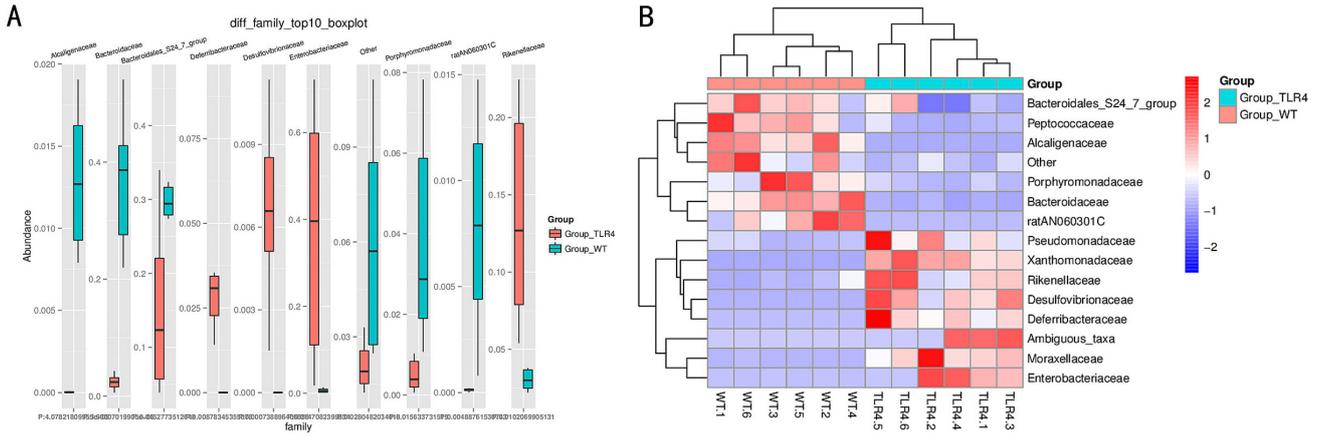


Fig. 6. Statistical analysis of microbial multivariate variables between TLR4-KO and WT mice following orally administered 1.5% DSS. (A,B) ANOVA statistical analysis of differences between TLR4-KO and WT mice following oral administration of 1.5% DSS at the family level.

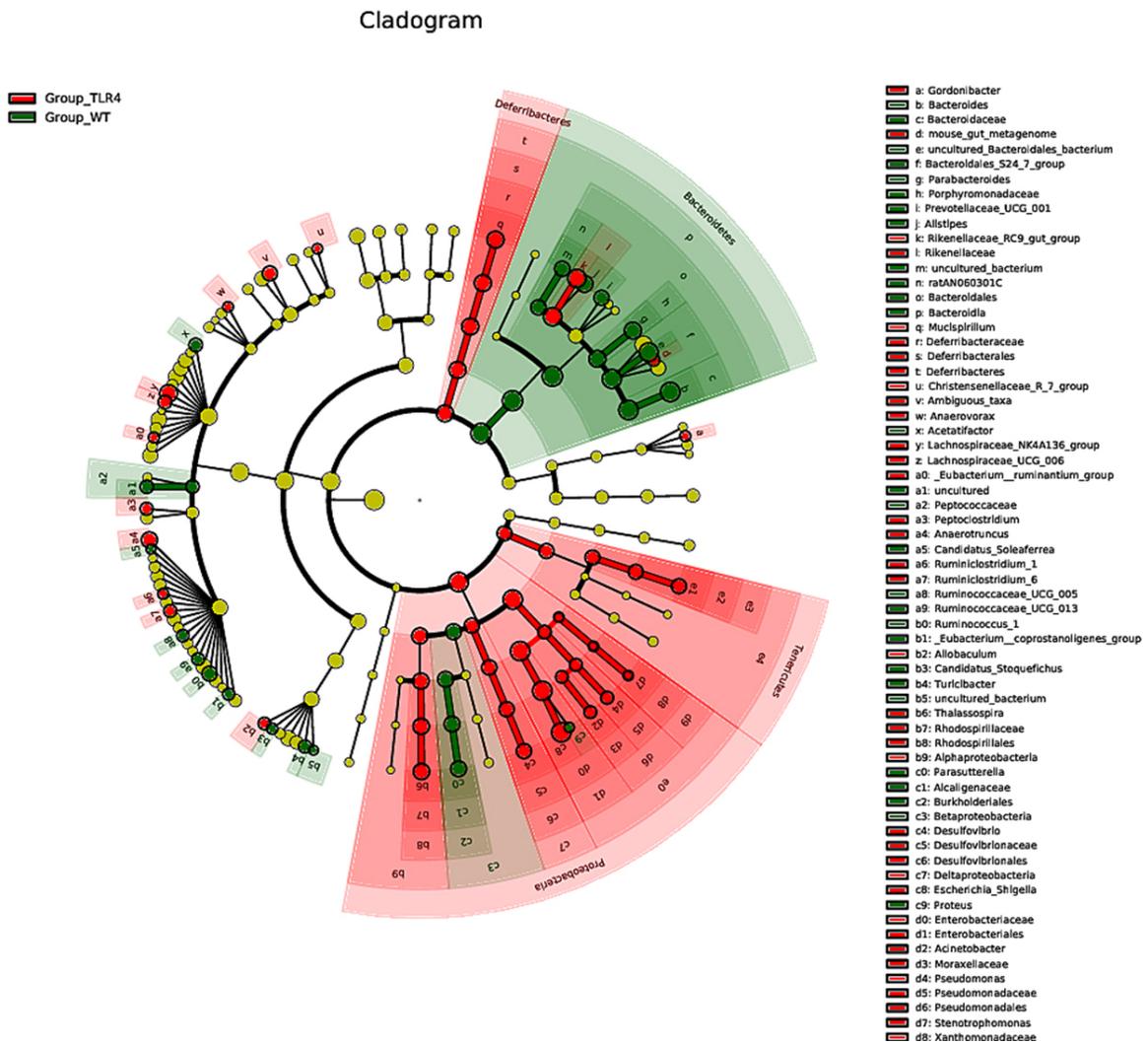


Fig. 7. Differential enrichment of multiple taxa in the TLR4-KO and WT control mice feces as per coupled with effect size measurements (LEfSe) analysis.

bleeding between TLR4 KO and WT mouse groups were compared. The data implied that TLR4 had a protective function against DSS-induced intestinal damage. Some studies have shown that TLR4 is the primary medium of LPS response both *in vitro* and *in vivo*. It has been established that TLR4 signaling exacerbates intestinal injury in mice undergoing DSS-induced colitis [34–36]. Contrary to prior research, our previous study found that moderately activating TLR4 signaling elevated inflammation and facilitated repair of the intestinal epithelium in DSS-induced colitis, both *in vitro* and *in vivo* [37]. Moderate activation of TLR4 signaling upregulated the levels of expression of repair factors PGE2 and GM-CSF [38,39] in the later stage of DSS-induced colitis. Meanwhile, Cario E *et al.* [40] reported that intestinal stem cells that could be found at the intestinal crypt base could promote the repopulation of the depleted crypt after intestinal epithelial injury, and this process is termed compensatory proliferation. This process may be involved in the moderate activation of the TLR4 signaling pathway.

Gut microbiota diversity is strongly linked to human health. The number of microbiota in the gut of healthy adults far exceeds the number of cells in the human body, which is a necessary condition for the normal functioning of the human gut. Research has increasingly demonstrated that gut microbiota dysfunction is the main cause of IBD [11]. Previous 16S rRNA sequencing analyses on the gut microbiota composition in IBD patients [41–44] demonstrated that α -diversity and the relative abundance of *Bacteroides* and *Firmicutes* were reduced, whereas the relative abundance of *Escherichia coli/Shigella*, *Enterobacter*, and *Fusobacterium* was increased in IBD patients as compared with healthy controls. Some of the gut microbiota was congruent with these research results. 16S rRNA gene sequencing in mice of this study demonstrated remarkable variation in the composition of gut microbiota, OTUs cluster, and β -diversity in WT and TLR4-KO mice after DSS treatment. However, no considerable variation was observed in α -diversity. In contrast with WT controls, the abundance of *Deferribacteres*, *Gammaproteobacteria*, *Tenerlcutes*, *Escherichia coli/Shigella*, *Enterobacter*, *Proteobacteria*, and *Rikenellaceae* was found to be increased in the gut microbiota of TLR4-KO mice, whereas the abundance of *Bacteroidetes* was considerably reduced at the five levels of phylogenetic hierarchy including phylum, class, order, family and genus. *Enterobacter* colonization in colitis could promote the absorption of neutrophils to the damaged mucosa [45]. In the process of intestinal infection, *Escherichia coli* could colonize the ileum and colon to cause damage to the intestinal mucosa. After colonization, *Shigella* is produced by *Escherichia coli* through intestinal epithelial cells (IECs). *Shigella* could stimulate the synthesis of inflammatory factors, the activation of immune cells, and the activation of complement, resulting in damage to the intestinal mucosa [46]. *Bac-*

teroidetes is a kind of probiotic, which performs a vital function in maintaining the homeostasis of the microbiota of the gut. The reduction of *Bacteroidetes* in the gut microbiota is considered a disorder, accompanied by IBD [47]. TLR4 ligands are synthesized by commensal microbiota [48]. Our previous study also found that when mice were orally administered neomycin, vancomycin, metronidazole (AVNM), and Polymyxin B (PMB) or ampicillin for four weeks, commensal-depleted mice depicted increased susceptibility to DSS-induced death and morbidity [37]. These data suggested that TLR4-KO might cause DSS-induced gut microbiota dysfunction in mice by up-regulating *Enterobacter*, *Escherichia coli/Shigella*, and down-regulating *Bacteroidetes*.

5. Conclusions

These results demonstrated severe susceptibility of TLR4-KO mice to DSS-induced colitis. This is consistent with the results of the research we have published. The data depicted the repairing effect of TLR4 on DSS-induced intestinal damage and that it can up-regulate CSF3, IL6, and CCL2 [7]. On this basis, this research depicted remarkable variation in the number of gut microbiota between TLR4-KO and WT mouse groups treated with 1.5% DSS via 16S rRNA gene sequencing. There were abundant *Enterobacter*, and *Escherichia coli/Shigella* in the gut microbiota of TLR4-KO mice, whereas the abundance of *Bacteroidetes* was considerably reduced at five phylogenetic levels as compared with WT controls. Collectively, moderate activation of TLR4 may perform some function in repairing the intestinal epithelium by up-regulating *Enterobacter*, *Escherichia coli/Shigella*, and down-regulating *Bacteroidetes*. The limitation of this study was that the gut microbiota screened by 16S rRNA sequencing was not been verified and the microbiota profiles of WT and TLR4-KO mice before 1.5% DSS administration was not be compared. Future research is planned to verify the various core gut microbiota that has been screened out between TLR4-KO and WT mice and establish a network map of intestinal microbiota with TLR4 as the core to repair IBD-related intestinal mucosal damage. The analysis of the relationship between downstream genes and key gut microbiota of TLR4 signaling in the repair of intestinal mucosal damage caused by DSS is also a key part of it.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article. The experimental data analyzed and displayed in the present manuscript are available from the corresponding author upon reasonable request.

Author Contributions

(I) Conception and design—HW, CL, ZQH; (II) Administrative support—HW; (III) Provision of study

materials—ZQH; (IV) Collection and assembly of data—YJS, CH; (V) Data analysis and interpretation—YJS, PQG; (VI) Manuscript writing—All authors; (VII) Final approval of manuscript—All authors.

Ethics Approval and Consent to Participate

All animal experiments conformed to the National Institute of Health Guide for the Care and Use of Laboratory Animals' (NIH Publication No. 85-23, National Academy Press, Washington, DC, revised 1996), with the approval of the Laboratory Animal Center of the Naval Medical University, Shanghai (No. 96-01).

Acknowledgment

Not applicable.

Funding

This work was supported by the National Key Basic Research Development Program of China (Grant No. 2015CB554000); the National Natural Science Foundation of China (Grant No. 82173005, 81573092, 81872046 & 81872559); and Shanghai Municipal Science and Technology Commission Research Program (Subject No. 17411951100).

Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at <https://doi.org/10.31083/j.fbl2808175>.

References

- [1] Adams SM, Bornemann PH. Ulcerative colitis. *American Family Physician*. 2013; 87: 699–705.
- [2] Torres J, Mehandru S, Colombel JF, Peyrin-Biroulet L. Crohn's disease. *Lancet* (London, England). 2017; 389: 1741–1755.
- [3] He X, Wei Z, Wang J, Kou J, Liu W, Fu Y, *et al.* Alpinetin attenuates inflammatory responses by suppressing TLR4 and NLRP3 signaling pathways in DSS-induced acute colitis. *Scientific Reports*. 2016; 6: 28370.
- [4] Geary RB. IBD and Environment: Are There Differences between East and West. *Digestive Diseases* (Basel, Switzerland). 2016; 34: 84–89.
- [5] Li Z, Zhang DK, Yi WQ, Ouyang Q, Chen YQ, Gan HT. NF-kappaB p65 antisense oligonucleotides may serve as a novel molecular approach for the treatment of patients with ulcerative colitis. *Archives of Medical Research*. 2008; 39: 729–734.
- [6] Tanaka K. Expression of Toll-like receptors in the intestinal mucosa of patients with inflammatory bowel disease. *Expert Review of Gastroenterology & Hepatology*. 2008; 2: 193–196.
- [7] Shi YJ, Hu SJ, Zhao QQ, Liu XS, Liu C, Wang H. Toll-like receptor 4 (*TLR4*) deficiency aggravates dextran sulfate sodium (DSS)-induced intestinal injury by down-regulating *IL6*, *CCL2* and *CSF3*. *Annals of Translational Medicine*. 2019; 7: 713.
- [8] Sun BL, Li WW, Wang J, Xu YL, Sun HL, Tian DY, *et al.* Gut Microbiota Alteration and Its Time Course in a Tauopathy Mouse Model. *Journal of Alzheimer's Disease: JAD*. 2019; 70: 399–412.
- [9] Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. *Nature Reviews. Microbiology*. 2021; 19: 55–71.
- [10] Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012; 486: 207–214.
- [11] Kostic AD, Xavier RJ, Gevers D. The microbiome in inflammatory bowel disease: current status and the future ahead. *Gastroenterology*. 2014; 146: 1489–1499.
- [12] Sartor RB. Microbial influences in inflammatory bowel diseases. *Gastroenterology*. 2008; 134: 577–594.
- [13] Ruiz E, Penrose HM, Heller S, Nakhoul H, Baddoo M, Flemington EF, *et al.* Bacterial TLR4 and NOD2 signaling linked to reduced mitochondrial energy function in active inflammatory bowel disease. *Gut Microbes*. 2020; 11: 350–363.
- [14] Cario E. Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. *Gut*. 2005; 54: 1182–1193.
- [15] Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, *et al.* Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *The Journal of Biological Chemistry*. 2003; 278: 8869–8872.
- [16] Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, *et al.* The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature*. 2001; 410: 1099–1103.
- [17] Trier JS. Mucosal flora in inflammatory bowel disease: Intraepithelial bacteria or endocrine epithelial cell secretory granules? *Gastroenterology*. 2002; 123: 955; author reply 956.
- [18] Philpott DJ, Girardin SE. Crohn's disease-associated Nod2 mutants reduce IL10 transcription. *Nature Immunology*. 2009; 10: 455–457.
- [19] Randhawa PK, Singh K, Singh N, Jaggi AS. A review on chemical-induced inflammatory bowel disease models in rodents. *The Korean Journal of Physiology & Pharmacology: Official Journal of the Korean Physiological Society and the Korean Society of Pharmacology*. 2014; 18: 279–288.
- [20] Bauer C, Diewell P, Mayer C, Lehr HA, Fitzgerald KA, Dauer M, *et al.* Colitis induced in mice with dextran sulfate sodium (DSS) is mediated by the NLRP3 inflammasome. *Gut*. 2010; 59: 1192–1199.
- [21] Eichele DD, Kharbanda KK. Dextran sodium sulfate colitis murine model: An indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis. *World Journal of Gastroenterology*. 2017; 23: 6016–6029.
- [22] Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell*. 2004; 118: 229–241.
- [23] Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis in mice. *Current Protocols in Immunology*. 2014; 104: 15.25.1–15.25.14.
- [24] Liu C, Zhang C, Mitchel REJ, Cui J, Lin J, Yang Y, *et al.* A critical role of toll-like receptor 4 (TLR4) and its' *in vivo* ligands in basal radio-resistance. *Cell Death & Disease*. 2013; 4: e649.
- [25] Yang C, Fujita Y, Ren Q, Ma M, Dong C, Hashimoto K. Bifidobacterium in the gut microbiota confer resilience to chronic social defeat stress in mice. *Scientific Reports*. 2017; 7: 45942.
- [26] Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* (Oxford, England). 2011; 27: 2194–2200.
- [27] Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology*. 2007; 73: 5261–5267.
- [28] Mount DW. Using the Basic Local Alignment Search Tool (BLAST). *CSH Protocols*. 2007; 2007: pdb.top17.

- [29] Blaxter M, Mann J, Chapman T, Thomas F, Whitton C, Floyd R, *et al.* Defining operational taxonomic units using DNA barcode data. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* 2005; 360: 1935–1943.
- [30] Bermon S, Petriz B, Kajènienè A, Prestes J, Castell L, Franco OL. The microbiota: an exercise immunology perspective. *Exercise Immunology Review.* 2015; 21: 70–79.
- [31] Nakatsu CH, Byappanahalli MN, Nevers MB. Bacterial Community 16S rRNA Gene Sequencing Characterizes Riverine Microbial Impact on Lake Michigan. *Frontiers in Microbiology.* 2019; 10: 996.
- [32] Burgueño JF, Fritsch J, González EE, Landau KS, Santander AM, Fernández I, *et al.* Epithelial TLR4 Signaling Activates DUOX2 to Induce Microbiota-Driven Tumorigenesis. *Gastroenterology.* 2021; 160: 797–808.e6.
- [33] Čuzić S, Antolić M, Ognjenović A, Stupin-Polančec D, Petrinić Grba A, Hrvačić B, *et al.* Claudins: Beyond Tight Junctions in Human IBD and Murine Models. *Frontiers in Pharmacology.* 2021; 12: 682614.
- [34] Qu Y, Li X, Xu F, Zhao S, Wu X, Wang Y, *et al.* Kaempferol Alleviates Murine Experimental Colitis by Restoring Gut Microbiota and Inhibiting the LPS-TLR4-NF- κ B Axis. *Frontiers in Immunology.* 2021; 12: 679897.
- [35] Zhang Y, Xu Y, Zhang L, Chen Y, Wu T, Liu R, *et al.* Licorice extract ameliorates hyperglycemia through reshaping gut microbiota structure and inhibiting TLR4/NF- κ B signaling pathway in type 2 diabetic mice. *Food Research International (Ottawa, Ont.).* 2022; 153: 110945.
- [36] Yang QY, Ma LL, Zhang C, Lin JZ, Han L, He YN, *et al.* Exploring the Mechanism of Indigo Naturalis in the Treatment of Ulcerative Colitis Based on TLR4/MyD88/NF- κ B Signaling Pathway and Gut Microbiota. *Frontiers in Pharmacology.* 2021; 12: 674416.
- [37] Shi YJ, Gong HF, Zhao QQ, Liu XS, Liu C, Wang H. Critical role of toll-like receptor 4 (TLR4) in dextran sulfate sodium (DSS)-Induced intestinal injury and repair. *Toxicology Letters.* 2019; 315: 23–30.
- [38] Egea L, McAllister CS, Lakhdari O, Minev I, Shenouda S, Kagnoff MF. GM-CSF produced by nonhematopoietic cells is required for early epithelial cell proliferation and repair of injured colonic mucosa. *Journal of Immunology (Baltimore, Md.: 1950).* 2013; 190: 1702–1713.
- [39] Miyoshi H, VanDussen KL, Malvin NP, Ryu SH, Wang Y, Sonnek NM, *et al.* Prostaglandin E2 promotes intestinal repair through an adaptive cellular response of the epithelium. *The EMBO Journal.* 2017; 36: 5–24.
- [40] Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK. Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *Journal of Immunology.* 2000; 164: 966–972.
- [41] Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, Gaynor EC, *et al.* Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. *Cell Host & Microbe.* 2007; 2: 204.
- [42] Darfeuille-Michaud A, Boudeau J, Bulois P, Neut C, Glasser AL, Barnich N, *et al.* High prevalence of adherent-invasive *Escherichia coli* associated with ileal mucosa in Crohn's disease. *Gastroenterology.* 2004; 127: 412–421.
- [43] Chassaing B, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology.* 2011; 140: 1720–1728.
- [44] Benjamin JL, Hedin CRH, Koutsoumpas A, Ng SC, McCarthy NE, Prescott NJ, *et al.* Smokers with active Crohn's disease have a clinically relevant dysbiosis of the gastrointestinal microbiota. *Inflammatory Bowel Diseases.* 2012; 18: 1092–1100.
- [45] Keith JW, Dong Q, Sorbara MT, Becattini S, Sia JK, Gjonbalaj M, *et al.* Impact of Antibiotic-Resistant Bacteria on Immune Activation and *Clostridioides difficile* Infection in the Mouse Intestine. *Infection and Immunity.* 2020; 88: e00362–19.
- [46] Lee KS, Jeong YJ, Lee MS. *Escherichia coli* Shiga Toxins and Gut Microbiota Interactions. *Toxins.* 2021; 13: 416.
- [47] Stojanov S, Berlec A, Štrukelj B. The Influence of Probiotics on the Firmicutes/Bacteroidetes Ratio in the Treatment of Obesity and Inflammatory Bowel disease. *Microorganisms.* 2020; 8: 1715.
- [48] Hacini-Rachinel F, Gomez de Agüero M, Kanjarawi R, Moro-Sibilot L, Le Luduec JB, Macari C, *et al.* Intestinal dendritic cell licensing through Toll-like receptor 4 is required for oral tolerance in allergic contact dermatitis. *The Journal of Allergy and Clinical Immunology.* 2018; 141: 163–170.