Influence of TRAIL Deficiency on Th17 Cells and Colonic Microbiota in Experimental Colitis Mouse Model

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ABSTRACT

Background: The abnormalities of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) are implicated in various autoimmune disorders and tumors. This study investigated the influence of TRAIL deficiency on Th17 cells and colonic microbiota in experimental colitis mouse model.

Methods: Mice were randomly divided into 4 groups: wild-type, TRAIL gene knock-out (*TRAIL*$^{-/-}$), wild-type colitis and *TRAIL*$^{-/-}$ colitis groups. Colitis was induced by oral administration of 3.5% dextran sulfate sodium (DSS) for 7 consecutive days. Mice were given scores for disease severity both clinically and histopathologically. Th17 cells in peripheral blood and mesenteric lymph nodes (MLNs) were assessed using flow cytometry. The expression levels of Th17 cell markers IL-17A and ROR-γt were evaluated by quantitative real-time polymerase chain reaction. The colonic samples were also analyzed for microbiota profile by 16s-rDNA gene sequencing on variable V4 region.

Results: Compared with wild-type counterparts, *TRAIL*$^{-/-}$ mice developed more severe colitis after DSS treatment. Colitis *TRAIL*$^{-/-}$ mice had increased proportion of Th17 cells and elevated mRNA expression levels of IL-17A and ROR-γt in peripheral blood and MLNs compared with colitis wild-type mice. In contrast to colitis wild-type mice, the composition of colonic microbiota was shifted in colitis *TRAIL*$^{-/-}$ mice, and was characterized by increased alpha diversity, increased TM7, deferribacteres and tenericutes, and decreased proteobacteria at the phylum level.

Conclusions: These findings suggested that TRAIL deficiency not only aggravated DSS-induced colitis, but also led to enhanced Th17 cell response and altered colonic microbiota composition.

Keywords: TRAIL deficiency; Th17 cells; Colonic microbiota; Experimental colitis mouse model. **[Am J Med Sci 2021;362 (2):188–197.]**

INTRODUCTION

Although the precise etiology of inflammatory bowel diseases (IBDs) is not fully clarified, it is generally believed that an inappropriate and persistent immune response against intestinal microbiota plays a key role in the pathogenesis of IBD.[1-4] The primary function of Th17 cells is to protect the host against pathogens of intestinal microbiota, which cannot be sufficiently handled by Th1 or Th2 cells.[5] In recent years, more and more studies have reported that the imbalance between Th17 cells and regulatory T cells (Tregs) contributes to the exacerbation of intestinal inflammation of IBD.[6-8] The proportion of Th17 cells and the mRNA expression levels of IL-17A and IL-17F were obviously elevated in the inflamed tissues and peripheral blood mononuclear cells (PBMCs) of active IBD patients when compared with the healthy controls.[9-10] The disease severity of UC patients was also correlated with IL-17A levels present in the PBMCs.[11] Consequently, the Th17 cell subset that releases IL-17 is progressively considered as a potent pro-inflammatory factor in the process of UC and CD.[9-11]

Intestinal microbiota has become the subject of intense focus over the last decade, as it is central in the development of gut immune system as well as in the maintenance of immune tolerance. There is increasing evidence that disturbances in gut microbiota may lead to abnormal proliferation and differentiation of Th17 cell population.[12] The germ-free or antibiotic-treated mice have significantly fewer Th17 cells in the absence of commensal bacteria than under normal conditions.[13] Low-dose penicillin pretreatment in mice exerts a unique protective effect against colitis induced by dextran sulfate sodium (DSS).[14] Further research suggested that low-dose penicillin causes specific changes to the gut microbiota, especially eradicates the segmented filamentous bacteria that are responsible for penicillin-induced suppression of IL-17 mRNA expression and Th17 cell differentiation.[14]

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) belongs to the TNF superfamily and

induces apoptosis in cells that express its cognate death receptors (human death receptors 4 and 5 or murine TRAIL-R).[15-16] Previous studies have reported that TRAIL is widely expressed in a variety of cells, such as monocytes, dendritic cells and T cells.[17-18] Mice with TRAIL gene knock-out (*TRAIL*$^{-/-}$) usually have severe defects in thymocyte apoptosis and are hypersensitive to collagen-induced arthritis and streptozotocin-induced diabetes, suggesting its pivotal role in the maintenance of central tolerance by negative selection of autoreactive thymocytes.[19] In contrast to the wild-type counterparts, *TRAIL*$^{-/-}$ mice manifest an earlier onset and a more severe form of experimental autoimmune encephalitis (EAE) disease, which are mainly attributed to the reduced apoptosis of Th1 cells.[20] In addition to the regulation of Th1 and Th2 cell polarization,[20-22] TRAIL can also influence the activity and proliferation of Th17 cells in experimental inflammatory arthritis and EAE mice.[23-25]

By reviewing the previous association studies of TRAIL and IBD, Brost et al. found a remarkable and significant lower expression of TRAIL in the surface epithelium of the IBD patients with active stage.[26] In addition, Begue et al. observed an upregulation of TRAIL in enterocytes in the IBD patients with remission stage.[27] Therefore, it can be inferred that the expression level of TRAIL in enterocytes may vary with the course e of disease in IBD patients. In vitro study demonstrated that TRAIL induced human intestinal epithelial cell apoptosis under inflammatory conditions, indicating that TRAIL contributes to the pathogenesis of gut inflammation.[27] Although chyuan et al. discovered that exogenous recombinant TRAIL significantly suppressed gut inflammation and reduced the severity of colitis in a DSS colitis model,[28] there were few studies on the relationship between TRAIL deficiency and intestinal microbiota in the context of IBD. Thus, we utilized *TRAIL*$^{-/-}$ mice to investigate the impact of TRAIL on Th17 cells and DSS-induced acute experimental colitis mouse model to study the intestinal microbiota composition. Our findings not only assist in the elucidation of IBD pathogenesis, but also can guide in the designing of therapeutic strategies for treating the disease.

METHODS

Animals and DSS-induced colitis

TRAIL$^{-/-}$ mice (C57BL/6 background) were generated using CRISPR/Cas9 method from Shanghai Biomodel Organism Science & Technology Development Co. Ltd (Shanghai, China). The *TRAIL*$^{-/-}$ genotype was verified by PCR (Supplementary Figure 1). No potential off-target events were detected in *TRAIL*$^{-/-}$mice. Male C57BL/6 wild-type mice were purchased from SLAC Laboratory Animal Co. Ltd (Shanghai, China). *TRAIL*$^{-/-}$ or wild-type mice of 6–8 weeks old were selected and raised in the same conditions for 1 week before DSS treatment. All mice used in the study were housed in the Laboratory Animal Center of Wenzhou Medical University under pathogen-free conditions and were monitored carefully for any signs of discomfort. All procedures were approved by the Institutional Animal Care and Use Committee at Wenzhou Medical University (Approval Document No. wydw2016−0215).

Colitis was induced in *TRAIL*$^{-/-}$ mice and wild-type mice using DSS according to the procedure that is described previously.[29] Briefly, DSS (molecular weight 36,000−50,000 kDa, MP Biomedicals, Canada) was dissolved in water at a final concentration of 3.5% and administered as drinking water for 7 days. On day 8, all mice were sacrificed by cervical dislocation. The peripheral blood, mesenteric lymph nodes (MLNs) and colons were collected. The intestinal pathology of each mouse was evaluated based on clinical disease activity index (DAI), colon length and histological score. These factors were recorded and scored for each mouse by two different investigators blinded to the treatment conditions.

Assessment of colitis severity

Macroscopic and histological analyses were performed as described previously.[29-30] postmortem, gross appearance, and colon length were evaluated. The removed colon tissue, fixed in formalin and embedded in paraffin, was stained with hematoxylin and eosin and the degree of colonic inflammation was graded based on the established criteria previously.[29]

Flow cytometry analyses

PBMCs were isolated directly from the peripheral blood of the orbital vein using lymphocyte separation medium according to the manufacturer's protocol (TBD science, Tianjin, China). Single-cell suspensions of MLNs were obtained by gently pressing the colonic tissues through a 70 μm cell strainer. Cell suspensions were washed twice in RPMI 1640 (Thermo Fisher Scientific, Waltham, MA, USA) and stored in media containing 10% fetal bovine serum (FBS) (Dakewe, Uruguay) on ice.

The cells were seeded at a density of 1×10^6 per well in 96-well plates and then cultured in RPMI 1640 medium supplemented with 1% PMA/ionomycin Mixture (250×) (MultiSciences, China) and 1% BFA/Monensin Mixture (250×) (MultiSciences, China) for 5 h. After that the cells were incubated with surface marker of FITC-labeled anti-mouse CD4 antibody (eBioscience, San Diego, CA, USA) for 30 min at 4 °C. Then the cells were washed and fixed overnight using fixation buffer (BioLegend, San Diego, CA, USA). On the second day, the cells were washed with intracellular staining perm wash buffer (BioLegend, USA), stained for 2 h at 4 °C with PE-conjugated anti-mouse IL-17A antibodies (eBioscience, San Diego, CA, USA) in permeabilization buffer (BioLegend, San Diego, CA, USA), and analyzed by flow cytometry (FCM). The stained mononuclear cells were analyzed on a FACS Aria II Cytometer using FlowJo software. Of all the mononuclear cells, $CD4^+IL\text{-}17A^+$ cells were considered to be Th17 cells.

Quantitative real time polymerase chain reaction

Total RNA was extracted from PBMCs or MLNs using Trizol reagent (Invitrogen, CA, USA). Reverse transcription was performed using PrimeScript reverse transcriptase (TaKaRa, Shiga, Japan) to obtain cDNA samples. The iTaq™ Universal SYBR® Green Supermix (Bio-Rad, Hercules, CA, USA) was employed to analyze the expression of mRNA. All primers for these reactions were synthesized by Shanghai Sangon Biotech Company (Shanghai, China). The prime sequences were as follows: murine β-actin 5′-GGCTGTATTCCCCTCCATCG-3′ (forward), 5′- CCAGTTGGTAACGCCATG-3′ (reverse); murine retinoic acid-related orphan receptor-γt (ROR-γt) 5′-CGCAGCCAGCAGTGTAAT-3′ (forward), 5′-TGACAGCATCTCGGGACAT-3′ (reverse); murine IL-17A 5′-GATGCTGTTGCTGCTGCTGAG-3′ (forward), 5′-GTGGAACGGTTGAGGTAGTCTGAG-3′ (reverse). The relative expression of the target genes was normalized to a standard housekeeping gene (β-actin) using the $2^{-\Delta\Delta CT}$ method.

DNA extraction and 16s-rDNA pyrosequencing

Transverse colonic tissues were collected from wild-type mice without DSS treatment (WC, $n = 8$), *TRAIL*$^{-/-}$mice without DSS treatment (TC, $n = 8$), wild-type mice with DSS treatment (WD, $n = 15$) and *TRAIL*$^{-/-}$mice with DSS treatment (TD, $n = 16$). The colonic samples were carefully cleaned and frozen immediately. DNA from the colonic tissues was extracted using the Omega E.Z.N.A.® Bacterial DNA Kit (Omega Bio-tek, Inc. Norcross, GA, USA). The 16s-rDNA gene was amplified using the Phusion® High-Fidelity PCR Master Mix (New England Biolabs, MA, USA). The PCR consisted of 30 cycles of denaturation (98 °C), annealing (55 °C), extension (72 °C), with final elongation (72 °C). Primers for the variable V4 region (515F to 806R) were used. Next, the PCR products were purified with Agencourt Ampure XP beads (Beckman Coulter, CA, USA) to remove the unspecific products. To validate these libraries, we determined the average molecular length using Agilent DNA 1000 reagents on Agilent 2100 bioanalyzer instrument (Agilent Technologies, CA, USA) and quantified the library by qPCR (EvaGreen®, Biotium, CA, USA). After applying MiSeq Reagent Kit (Illumina, CA, USA), the qualified libraries were finally paired-end sequenced on the Illumina MiSeq System, with the sequencing strategy of PE250.

Bioinformatics and statistical analyses

Body weight change, colon length, DAI scores, histological scores, the proportion of $CD4^+$and $CD4^+$IL-$17A^+$ *T* cells as well as qRT-PCR results are expressed as the means ± standard deviation (SD). Statistical comparisons were performed using one-way ANOVA. Statistical tests were evaluated with GraphPad Prism Software 5.0 (GraphPad Software, USA). Probabilities of $P<0.05$ or as indicated were considered to be statistically significant.

The raw data from 16s-rDNA pyrosequencing were filtered to obtain the clean reads by eliminating the adapter pollution and low quality data, and then the paired-end reads with overlap were merged to tags.[31] The tags were then clustered to OTU (Operational Taxonomic Unit) at 97% sequence similarity by scripts of software USEARCH (v7.0.1 090).[32] OTU representative sequences were taxonomically classified using Ribosomal Database Project (RDP) Classifier v.2.2 trained on the database Greengene_201 3_5_99, using 0.6 confidence values as cutoff. Based on the OTU abundance, it was listed from each group, and Venn diagram was drawn by VennDiagram software R (v3.1.1). Principal component analysis (PCA) was used to construct 2-D graph by package 'ade4' of software R (v3.1.1). Alpha diversity was analyzed based on OTU and taxonomic ranks. Wilcoxon test was applied to compare the differences in analysis between the groups of samples at the phylum, class, order, family, genus and species levels. *P* value was adjusted by Benjamini-Hochberg false discovery rate correction function 'p.adjust' in the stats package of R (v3.1.1).

RESULTS

Comparison of clinical manifestations and severity of colitis between *TRAIL*$^{-/-}$ and wild-type mice after DSS treatment

Mice were randomly assigned to 4 groups, including wild-type, *TRAIL*$^{-/-}$, wild-type colitis and *TRAIL*$^{-/-}$ colitis ($n = 20$/group). The *TRAIL*$^{-/-}$ and wild-type mice showed no weight loss without DSS treatment (Fig. 1A). In contrast, the mice in both *TRAIL*$^{-/-}$ and wild-type groups fed with DSS exhibited acute colitis. However, the percentage of weight loss was significantly higher in the *TRAIL*$^{-/-}$ colitis mice compared to the wild-type colitis mice (Fig. 1B). The percentage of weight loss after DSS administration was 28.34 ± 3.02 in *TRAIL*$^{-/-}$ colitis mice, and 17.42 ± 3.24 in wild-type colitis mice ($P<0.001$). In addition, DSS treatment demonstrated a significantly higher DAI and a significant reduction in colon length in *TRAIL*$^{-/-}$ mice compared with wild-type mice (all $P<0.01$), (Fig. 1B, C). According to the histology scoring criteria, the colon histopathologic scores of *TRAIL*$^{-/-}$ and wild-type mice without DSS treatment were both 0, which showed no significant differences in hematoxylin and eosin staining between the two groups (Fig. 1D). And it was in agreement with the previous reports.[19] However, after treatment with DSS, *TRAIL*$^{-/-}$ mice exhibited much more severe colonic inflammation compared with wild-type mice ($P<0.001$), (Fig. 1D, E). Taken together, these observations indicated that TRAIL deficiency aggravated DSS-induced colitis.

Effect of trail deficiency on Th17 cells after dss treatment

In concordance with the earlier studies on colitis mice,[33,34] the percentage of Th17 cells, together with the

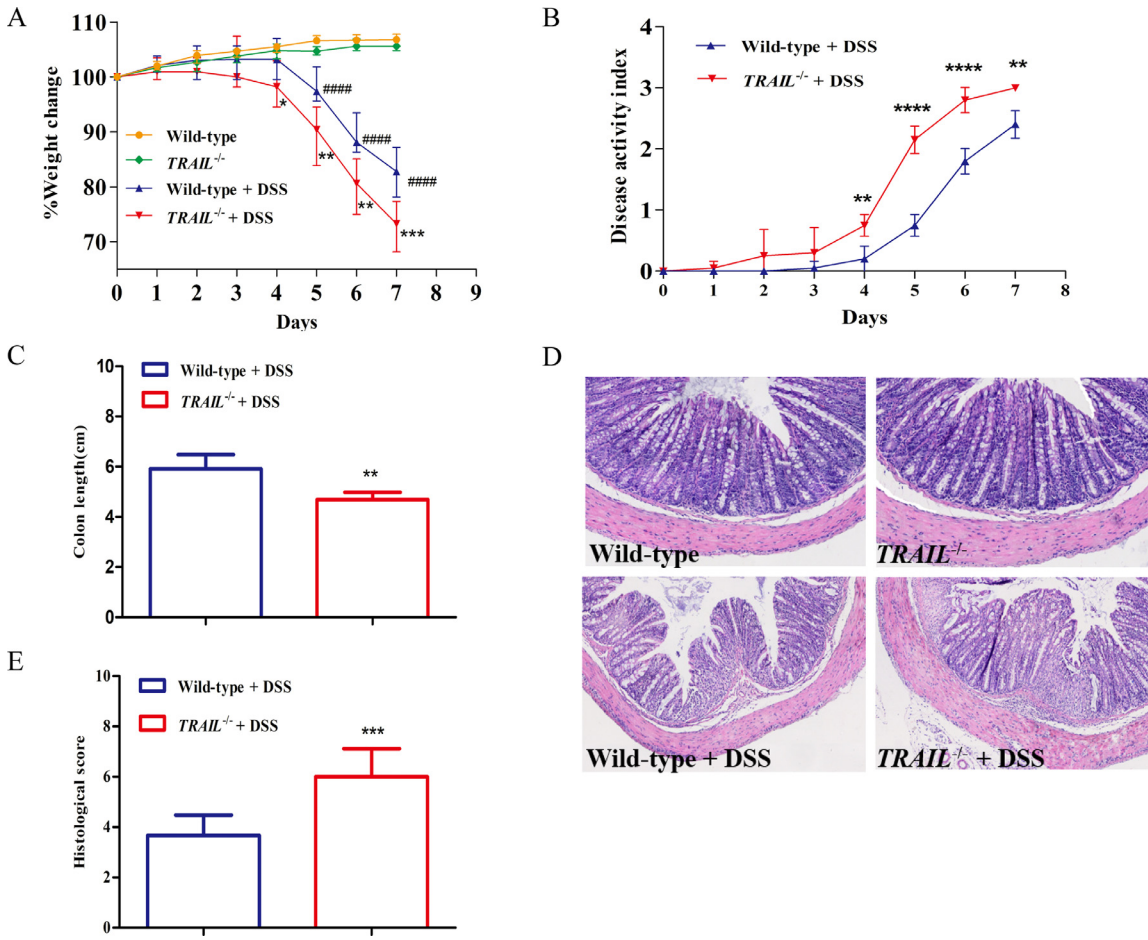

FIGURE 1. *TRAIL*$^{-/-}$mice displayed more severe acute colitis after administration of DSS. *TRAIL*$^{-/-}$ and wild-type mice were exposed to 3.5% DSS in drinking water for 7 consecutive days ($n = 20$). Colitis severity was assessed by weight loss (A), disease activity index (B), colon length (day 8) and (C) hematoxylin and eosin histology (day 8) (D, E). All data were expressed as means ± standard deviation (SD).

mRNA levels of Th17 cell-related inflammatory cytokine IL-17A and the key transcription factor ROR-γt, in PBMCs and MLNs of wild-type colitis mice were significantly higher compared to normal wild-type mice (all $P<0.05$), (Fig. 2, 3). The percentage of Th17 cells, as well as the mRNA levels of IL-17A and ROR-γt in PBMCs and MLNs were almost identical between *TRAIL*$^{-/-}$ and wild-type mice under non-inflammatory conditions (all $P>0.05$), (Fig. 2, 3). However, the proportions of CD4$^+$ *T* cells were significantly higher in PBMCs and MLNs from *TRAIL*$^{-/-}$ colitis mice than those from wild-type colitis mice (both $P<0.001$), (Fig. 2A, B). *TRAIL*$^{-/-}$ colitis mice also displayed a significantly higher percentage of CD4$^+$IL-17A$^+$ Th17 cells in PBMCs and MLNs ($P<0.001$ and $P<0.0001$, respectively), (Fig. 2C, D). As described in Fig. 3, the mRNA levels of IL-17A and ROR-γt were significantly elevated in both PBMCs and MLNs from *TRAIL*$^{-/-}$ colitis mice compared with those from wild-type colitis mice (all $P<0.01$). These data suggested that TRAIL deficiency promoted the number and activation of Th17 cells in DSS- induced colitis.

Effect of trail deficiency on the composition of colonic microbiota in mice with or without DSS treatment

A total of 4018,733 sequence tags and 2014 OTUs have been identified in all the 47 colonic samples. The results showed that proteobacteria and firmicutes were the most abundant in the colons of mice at the phylum level, followed by defferibacteres and tenericutes (Fig. 4). Venn diagram showed the number of OTUs in each group (Fig. 5A). PCA analyses showed that DSS treatment contributed to the shift of microbiota composition (Fig. 5B).

In the mice without DSS treatment (WC and TC), there was no difference in alpha diversity index between two groups ($P>0.05$). However, the relative abundances of some bacteria were significantly altered in the TC group (Supplementary Table 1). Notably, some of these bacteria with differential abundance were also increased/decreased in TD group compared with WD group. Of these, TM7 and deferribacteres at phylum level, TM7−3 and deferribacteres at class level, deferribacteraceae

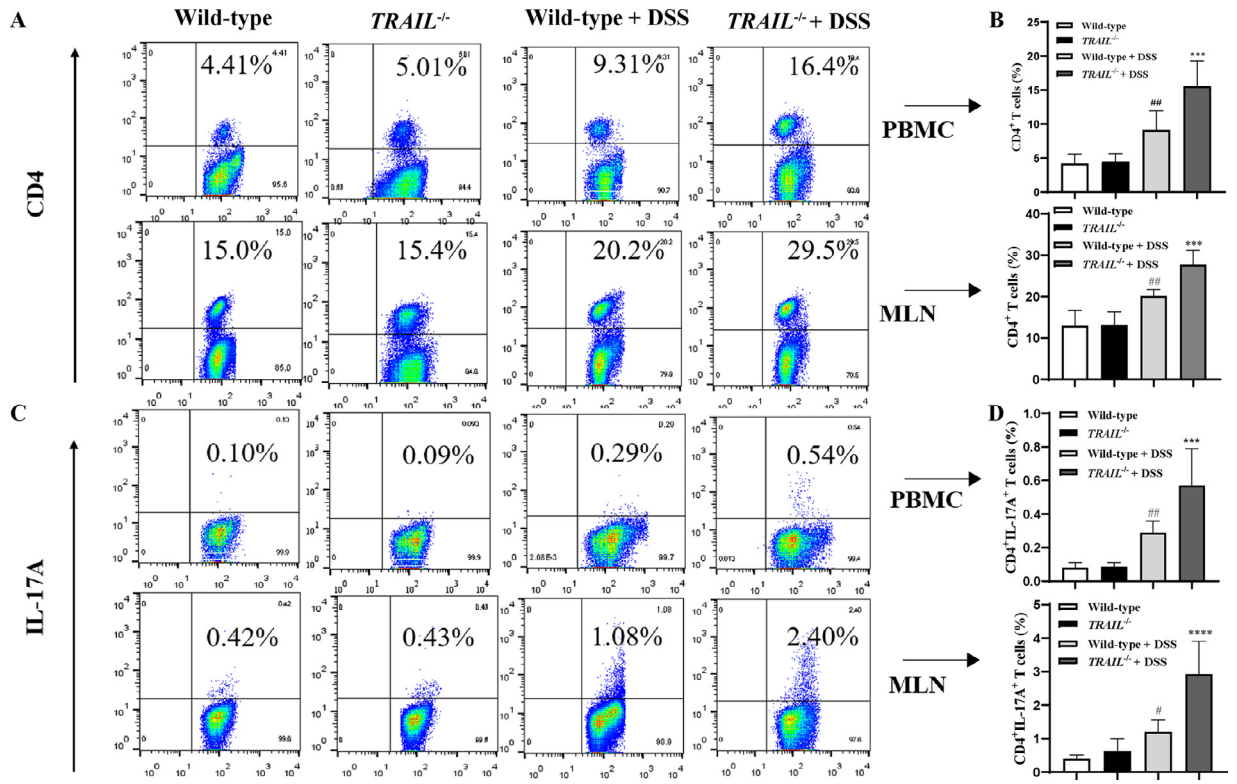

FIGURE 2. *TRAIL*$^{-/-}$ mice showed enhancement of both CD4$^+$ and CD4$^+$IL-17A$^+$ *T* cells in PBMCs and MLNs after colitis. Mononuclear cells from PBMCs and MLNs were stained for FITC-labeled anti-mouse CD4 and PE-conjugated anti-mouse IL-17A antibodies and then analyzed by flow cytometry. A representative experiment was shown in panels (A) and (C), indicating the percentage of CD4$^+$ *T* cells and CD4$^+$IL-17A$^+$T cells, respectively. The proportion of CD4$^+$ *T* cells and Th17 cells means ± standard deviation (SD) from each group was depicted in panels (B) and (D).

and F16 at family level, mucispirillum at genus level and mucispirillum schaedleri at species level were decreased in TC group compared with WC group, while paraprevotellaceae at family level and cytophaga at genus level were increased in TC group.

In mice with DSS treatment (WD and TD), Shannon diversity index was higher in TD group than WD group ($P = 0.041$), while Simpson diversity index was lower in TD group compared with WD group ($P = 0.042$). This indicated that TD group had a higher alpha diversity than WD group (Fig. 6). The relative abundances of specific bacteria were also altered in the *TRAIL*$^{-/-}$ mice compared to wild-type mice (Supplementary Table 2). At the phylum level, for instance, TM7, deferribacteres and tenericutes were significantly increased whereas proteobacteria was decreased in TD group compared with WD group. The relative change of bacteria between the two groups was illustrated in Fig. 7. Interestingly, TM7 phylum, deferribacteres phylum, mucispirillum genus and F16 family that are increased in TD group were initially decreased in TC group compared with WC group.

DISCUSSION

The present study showed that *TRAIL*$^{-/-}$ mice were developed normally under normal conditions. Upon DSS induction, the mice developed more severe colitis than wild-type counterparts. This finding was in agreement with the conclusions drawn from other mice models of autoimmune disorders, including experimental autoimmune encephalomyelitis and autoimmune arthritis.[18-20] Our study further revealed that the percentages of Th17 cells in PBMCs and MLNs were significantly elevated in *TRAIL*$^{-/-}$ mice compared with wild-type counterparts after treatment with DSS. The mRNA expression levels of IL-17A and ROR-γt in PBMCs and MLNs were also significantly higher in colitis *TRAIL*$^{-/-}$ mice than in colitis wild-type mice. These findings suggested that TRAIL deficiency conferred an increase in the number and activation of Th17 cells and aggravated the DSS-induced colitis.

The results on experimental inflammatory arthritis mice suggested that, exogenous TRAIL treatment after disease onset not only ameliorated the severity of disease, but also reduced the number of Th17 cells and the mRNA expression levels of IL-17 in inflammatory joint tissues.[24] In contrast to the vehicle-treated mice, the proportion of Annexin *V*$^+$ IL-17$^+$cells in inflammatory joint tissues was significantly enhanced in exogenous TRAIL-treated mice. This in turn suggested that the role of TRAIL in experimental inflammatory arthritis mainly depends on regulation of apoptosis of Th17 cells.[24]

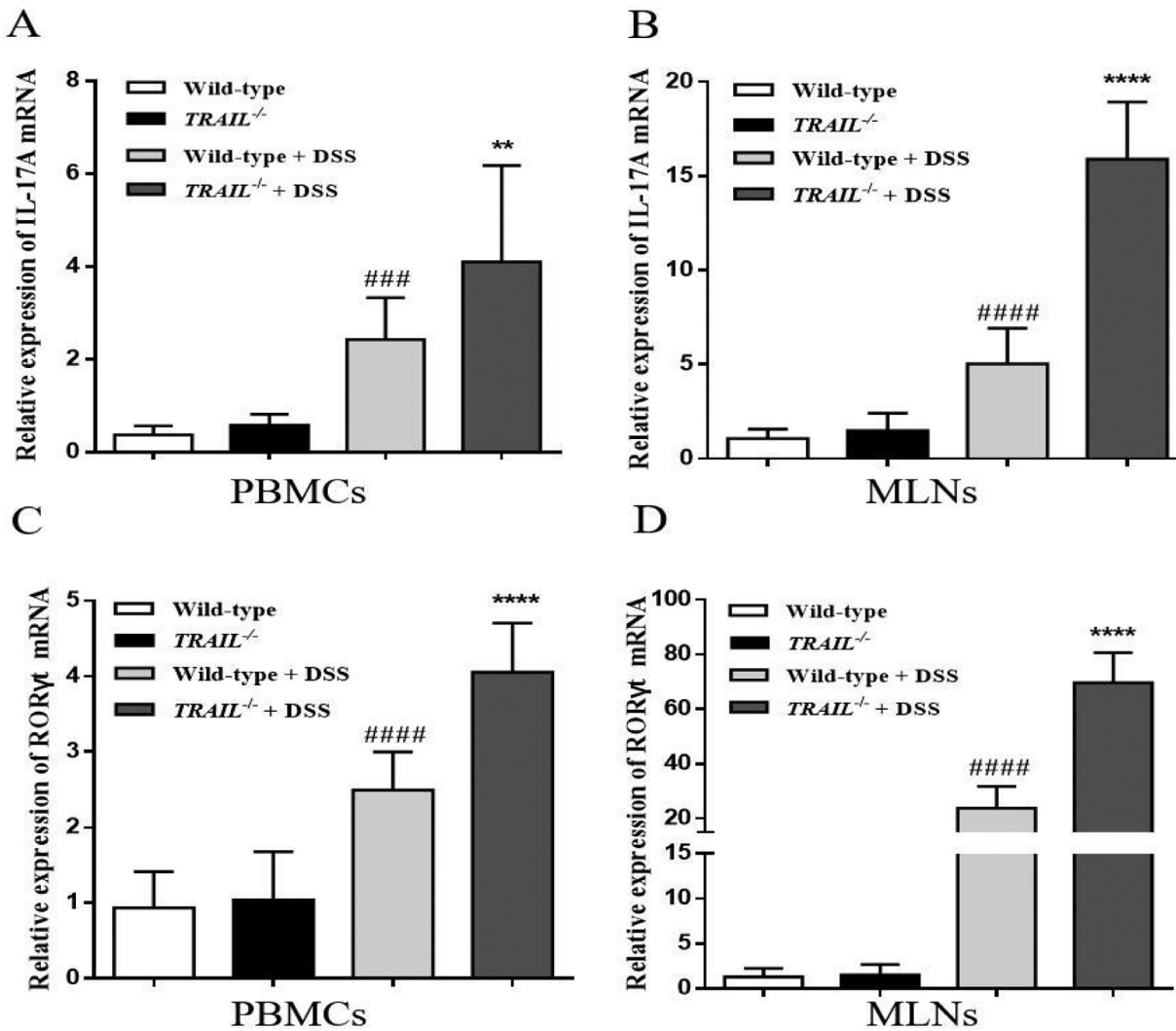

FIGURE 3. Th17 cells were increased in number and showed enhanced function in *TRAIL*$^{-/-}$ colitis mice. PBMCs and MLNs from *TRAIL*$^{-/-}$ colitis or wild-type counterparts were obtained to examine the mRNA levels of IL-17A and ROR-γt by real-time PCR. The results were expressed as means ± standard deviation (SD) for each group.

Notably, convincing evidence has shown that TRAIL directly inhibited the activation of target T cells via apoptotic-independent pathway.[25, 35-36] Chyuan et al. found that no significant cell apoptosis was induced among isolated CD4$^+$ *T* cell after being treated with different doses of TRAIL. Moreover, adoptive transfer of TRAIL-treated T cells reduced development of bowel inflammation and colitis in a T-cell transfer colitis model, indicating that TRAIL directly suppressed colitogenic T-cell activation and prevented the subsequent development of autoimmune colitis.[25] Besides, soluble TRAIL inhibited the proliferation of diabetogenic splenic T cells isolated from non-obese diabetic (NOD) mice by suppression of IL-2 production and cell cycle progression, but do not induce apoptosis of these T cells. This inhibition can be reversed in the presence of exogenous IL-2.[35] Additionally, TRAIL-induced hypoproliferation of T cells occurred due to the downregulation of cyclin-dependent kinase 4 and blockade of cell cycle progression.[36] Thus, we gussess that the effect of TRAIL deficiency on the number and activation of Th17 cells during colitis may attribute to some apoptotic-independent pathways.

To date, several studies have demonstrated that the interaction between gut microbiota and CD4$^+$ *T* cell subsets remained to be dynamic and interdependent, with each affecting the composition or function of the other.[37-38] For example, the segmented filamentous bacteria were sufficient to induce Th17 cells probably through stimulating the production of early inflammatory marker serum amyloid A in the lamina propria, while Th17 cells are almost absent in the gut mucosa of newborns and germ-free mice.[38-41]

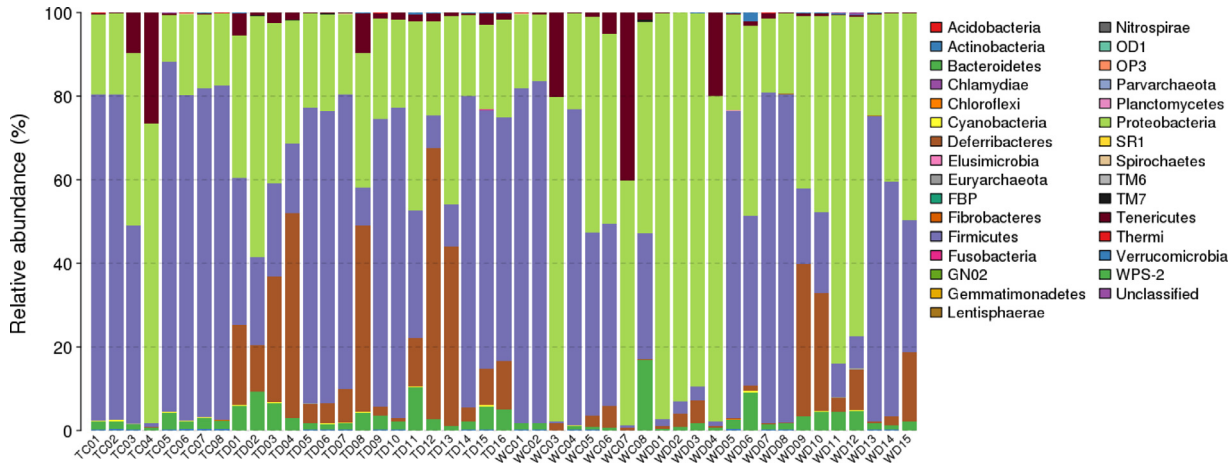

FIGURE 4. The relative abundance of bacteria at the phylum level in each colonic tissue sample from *TRAIL*$^{-/-}$ and wild-type mice with or without DSS treatment. WC=wild-type mice without DSS treatment, TC=*TRAIL*$^{-/-}$ mice without DSS treatment, WD=wild-type mice with DSS treatment, TD=*TRAIL*$^{-/-}$ mice with DSS treatment.

Additionally, immunodeficient Rag1 gene knock-out (*Rag1*$^{-/-}$) mice showed a significant increase in the microbial diversity with age compared to wild-type mice, highlighting that the adaptive immune system plays an important role in modulation of gut microbiota composition.[42]

It is widely accepted that the intestinal microflora dysbiosis with altered bacterial composition and loss of diversity were considered as main characteristics, which promoted the occurrence and development of IBD.[43] The present study indeed provided evidence that TRAIL deficiency resulted in an increased alpha diversity of microbiota in the DSS-induced colitis. Compared with colitis wild-type mice, colitis *TRAIL*$^{-/-}$ mice demonstrated a higher phylum abundance of TM7, deferribacteres and tenericutes, but a lower abundance of proteobacteria. Similarly, Kuehbacher et al. demonstrated a higher diversity of TM7 phylotypes in CD patients than in UC or non-IBD patients.[43] The study also further demonstrated that the TM7 bacteria in CD patients had more frequent base substitution, as this was mainly responsible for their resistance to antibiotics.

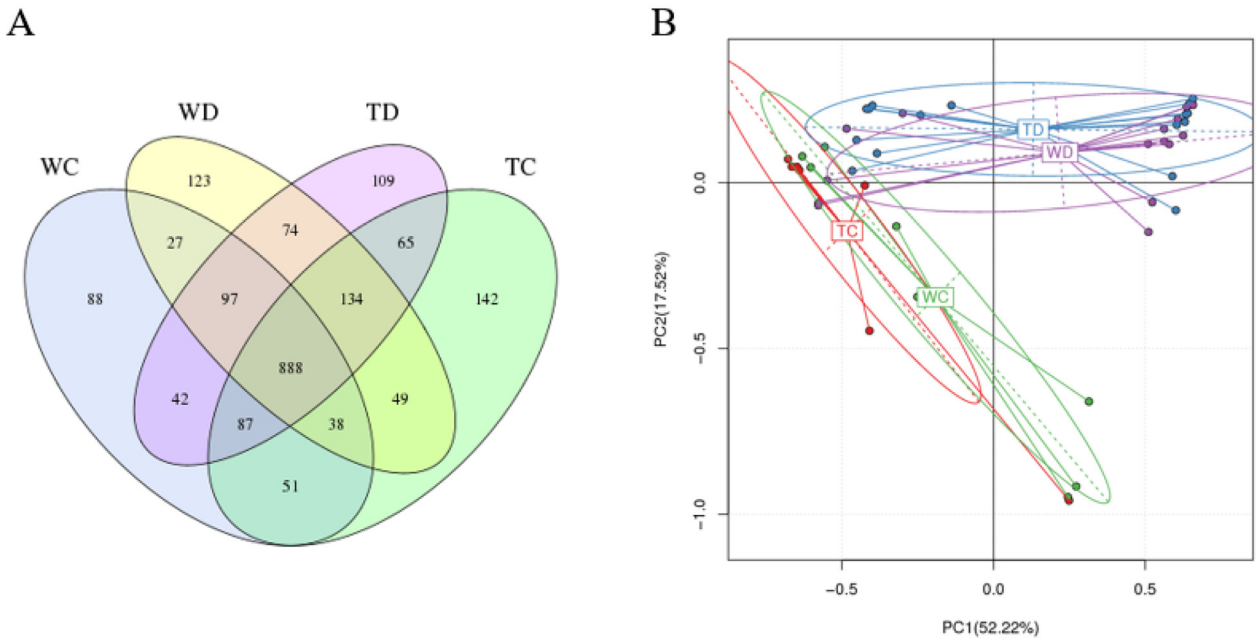

FIGURE 5. Overview of microbiota based on OTUs in *TRAIL*$^{-/-}$ and wild-type mice with or without DSS treatment. **A**: Venn diagram of OTUs in the four groups. **B**: Principal component analysis of microbiota from the four groups based on OTUs. WC=wild-type mice without DSS treatment, TC=*TRAIL*$^{-/-}$ mice without DSS treatment, WD=wild-type mice with DSS treatment, TD=*TRAIL*$^{-/-}$ mice with DSS treatment.

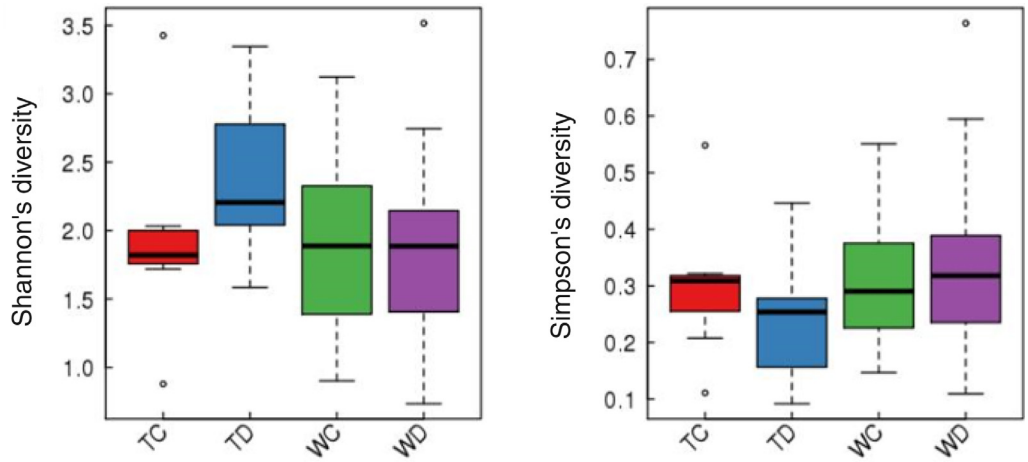

FIGURE 6. Boxplots of alpha diversity parameters (shannon's diversity and simpson' diversity) of microbiota in *TRAIL*$^{-/-}$ and wild-type mice with or without DSS treatment. Shanon diversity index in TD group was significantly higher than WD group ($P = 0.041$), while Simpson diversity index in TD group was significantly lower than WD group ($P = 0.042$), indicating that TD group had a higher alpha diversity than WD group. WC=wild-type mice without DSS treatment, TC=*TRAIL*$^{-/-}$ mice without DSS treatment, WD=wild-type mice with DSS treatment, TD=*TRAIL*$^{-/-}$ mice with DSS treatment.

The above results suggested that TM7 bacteria may facilitate a pro-inflammatory shift of intestinal microbiota in IBD patients.

Interestingly, our findings described above were basically consistent with the previous study on pig with experimental colitis, wherein an increase in deferribacteres and proteobacteria and a decrease in tenericutes were also observed.[44] As a mucus-dwelling commensal organism, deferribacteraceae is increased during inflammation and functions as pathogens in many diseases, such as DSS-induced colitis.[45-46] According to the available research data, proteobacteria is the most dominant phyla in the colonic tissues of mice. Lepage et al. reported that the patients with UC exhibited more abundance of proteobacteria than their healthy twins.[47] In our study, we noted that the proteobacteria was also augmented after treatment with DSS (WC versus WD group). Due to TRAIL deficiency, this microbe was

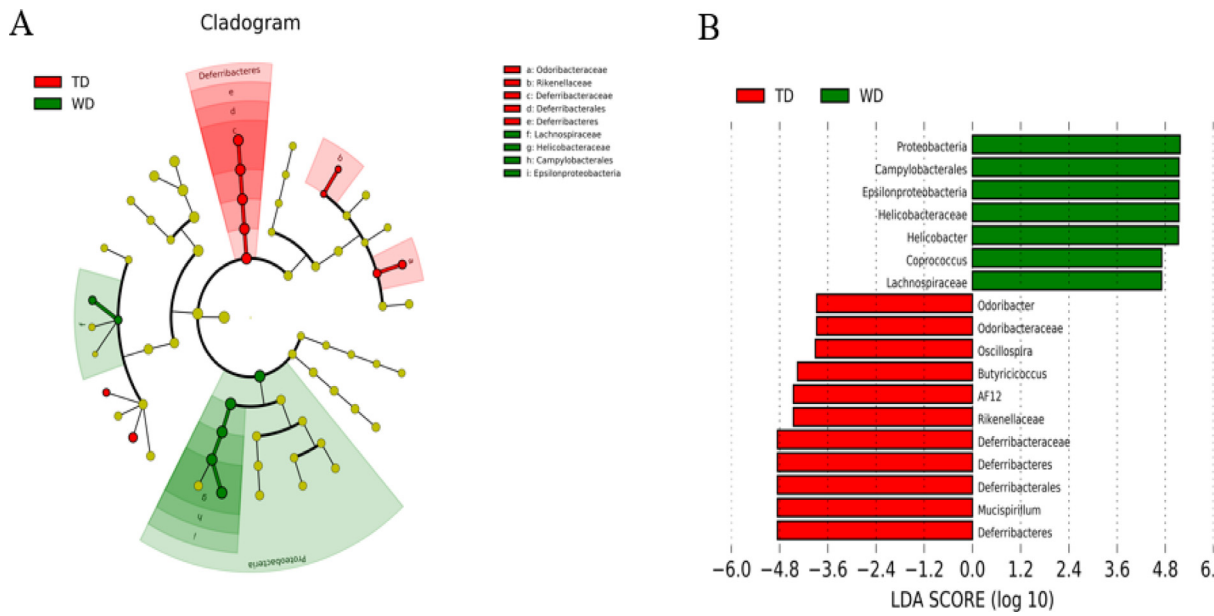

FIGURE 7. Differential microbes between TD and WD groups. **A**: Cladogram showed increased phylogenetic distribution of colonic microbes in TD group (red) and WD group (green) by LEfSe analysis. The yellow notes represent the microbes that do not show any difference in the groups. **B**: Linear discriminant analysis (LDA) scores represent microbes with significant difference between the TD and WD groups. WD=wild-type mice with DSS treatment, TD=*TRAIL*$^{-/-}$ mice with DSS treatment.

reduced in colitis model (TD versus WD group). Thus, an in-depth study to elucidate the underlying mechanisms through which TRAIL deficiency affects the proteobacteria in the DSS-induced colitis is warranted.

In addition, the increase of paraprevotellaceae (family level) and cytophaga (genus level) in DSS-induced colitis that resulted from TRAIL deficiency (TD versus WD group), has already been existed before administration of DSS. Ivanov et al. examined the composition of microbiota isolated from the cecum of Th17 cell-sufficient mice and Th17 cell-deficient mice. Consequently, the presence of Th17 cells in the small intestinal lamina propria was closely correlated with the number of cytophaga-flavobacte-bacteroidetes (CFB) phylum in all the subgroups, suggesting that this phylum was directly implicated in the differentiation of Th17 cells.[13] Our findings demonstrated that there was a potential link among TRAIL, intestinal microbiota and immunity. Further studies are needed to verify the function of intestinal flora and the cause for altered intestinal flora in *TRAIL*$^{-/-}$ mice.

However, the four microbes (TM7 phylum, mucispirillum genus, deferribacteres phylum and F16 family) were increased in TD group compared with WD group and were significantly decreased in TC group when compared with WC group. These microbes might be of interest for causality study because their *TRAIL* knockout-related changes were abundant and occurred in normal housing conditions, i.e., independent of DSS treatment. Of these, mucispirillum is a genus of the phylum deferribacteres and has been considered to be an indicator of DSS treatment.[46-47] In addition, mucispirillum is physically associated with the secreted mucus layer,[48] and may act as a pathogen to cause disease in certain cases.[49]

CONCLUSIONS

In summary, this study emphasized a protective role of TRAIL in the pathogenesis of IBD and also provided novel evidence that TRAIL deficiency aggravated DSS-induced colitis probably by enhanced Th17 response and altered colonic microbiota. Moreover, since TRAIL receptor agonists have drawn considerable attention for the treatment of tumors in several clinical trials,[21] our study also highlighted TRAIL as a potential therapeutic target for the treatment of IBD.

AUTHORS' CONTRIBUTIONS

Prof YJ conceived and designed the experiments; Dr DPL and Dr YLJ performed the experiments and wrote the paper. Dr DYH analyzed the data; Dr SJY made appraisals of manuscript for important content. All authors have read and approved the manuscript.

FUNDING STATEMENT

This work was supported by grants of Natural Science Foundation of Zhejiang Province (Grant number: LY15H030018, LY16H160055, LY17H030011, LY18H030009), and the Wenzhou Municipal Science and Technology Bureau (Grant number: Y20160102, Y20170314, Y20170062).

CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.amjms.2021.04.011.

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Submitted May 3, 2020; accepted April 21, 2021.

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