**Enterococcus durans** TN-3 Induces Regulatory T Cells and Suppresses the Development of Dextran Sulfate Sodium (DSS)-Induced Experimental Colitis

Toshihiro Kanda¹, Atsushi Nishida¹ *, Masashi Ohno¹, Hirotugu Imaeda¹, Takashi Shimada², Osamu Inatomi¹, Shigeki Bamba¹, Mitsushige Sugimoto¹, Akira Andoh¹

¹ Department of Medicine, Shiga University of Medical Science, Seta-Tsukinowa, Otsu, Shiga, Japan, 2 Central Research Laboratories, Nichinichi Pharmaceutical Corporation Ltd., Tominaga, Iga, Mie, Japan

* atsuda@belle.shiga-med.ac.jp

**Abstract**

**Background and Aims**

Probiotic properties of *Enterococcus* strains have been reported previously. In this study, we investigated the effects of *Enterococcus (E.) durans* TN-3 on the development of dextran sulfate sodium (DSS) colitis.

**Methods**

BALB/c mice were fed with 4.0% DSS in normal chow. Administration of TN-3 (10mg/day) was initiated 7days before the start of DSS feeding. Mucosal cytokine expression was analyzed by real time-PCR and immunohistochemistry. The lymphocyte subpopulation were analyzed by flow cytometry. The gut microbiota profile was analyzed by a terminal-restriction fragment length polymorphism method (T-RFLP).

**Results**

The disease activity index and histological colitis score were significantly lower in the DSS plus TN-3 group than in the DSS group. The mucosal mRNA expression of proinflammatory cytokines (IL-1β, IL-6, IL-17A and IFN-γ) decreased significantly in the DSS plus TN-3 group as compared to the DSS group. The proportion of regulatory T cells (Treg cells) in the mucosa increased significantly in the DSS plus TN-3 group as compared to the DSS group. Both fecal butyrate levels and the diversity of fecal microbial community were significantly higher in the TN-3 plus DSS group than in the DSS group.

**Conclusions**

*E. durans* TN-3 exerted an inhibitory effect on the development of DSS colitis. This action might be mediated by the induction of Treg cells and the restoration of the diversity of the gut microbiota.
Introduction

Inflammatory bowel disease (IBD), such as ulcerative colitis (UC) and Crohn’s disease (CD), are chronic intestinal inflammatory disorders of unknown etiology [1, 2]. Recent studies suggested that a dysfunction of the host immune response against dietary factors and the commensal bacteria plays an important role in the pathogenesis of IBD [3–7].

Probiotics are live microorganisms that provide beneficial effects to the host when administered in adequate amounts [8]. They exert these effects by modulating the gut microbiota and promoting mucosal barrier functions and resistance to pathogens. Now, probiotics are considered to be a therapeutic option for inducing or maintaining clinical remission of IBD.

Most of probiotics consist of Lactobacillus spp., Bifidobacterium spp. and Enterococcus spp. [9]. Among Enterococcus spp., Enterococcus durans (E. durans) has been reported to exert various probiotic effects. For example, E. durans strain 6HL, isolated from the vagina of healthy women, possesses the ability to inhibit the growth of pathogenic microorganisms [10]. Avram-Hananel et al. reported that E. durans strain M4-5, isolated from the human colon, improved intestinal inflammation [11]. Kondoh et al. reported that E. durans strain TN-3, isolated from deep seawater, effectively suppressed dermal eosinophil accumulation in allergen-primed mice [12].

Several animal models of experimental colitis induced by chemical agents have been employed to investigate the pathophysiology of IBD [13, 14]. DSS colitis model is one of the widely used models owing to the reproducibility. DSS colitis is morphologically characterized by epithelial cell damage, ulceration, submucosa edema, and the infiltration of granulocytes and mononuclear immune cells. Therefore, DSS colitis model is considered to exhibit features of relevance of human ulcerative colitis [15–17]. In this study, we used the regimen of a continuous administration of DSS to examine the effect of preventive treatment of TN-3 on the induction of acute phase of intestinal injury.

Here, we investigated the effects of E. durans TN-3 on dextran sulfate sodium (DSS)-induced colitis to explore its therapeutic potential for IBD patients. We further analyzed the effects of E. durans TN-3 on the mucosal lymphocyte subpopulation and fecal levels of short-chain fatty acids.

Materials and Methods

Experimental animals and induction of colitis

BALB/cAcl mice (Six to eight week-old females) were purchased from CLEA Japan (Tokyo, Japan). They were acclimatized for one week before the experiment, and were housed individually in a room maintained at 22°C under a 12-h day/night cycle throughout the experiments. They were allowed free access to rodent chow (MF; Oriental Yeast Co., Ltd, Tokyo, Japan) and drinking water. Experimental colitis was initiated by the oral administration of 4% DSS (molecular weight 5000; Wako Pure Chemical Industries, Ltd, Osaka, Japan) mixed with normal chow. Mice were divided into 4 groups; control mice, TN-3-treated mice, 4% DSS-treated mice, and 4% DSS plus TN-3-treated mice. For the examination of preventive effect of TN-3 on the colitis, TN-3 (10mg/day in 0.3ml phosphate buffered saline (PBS)) was administrated by oral gavage 7 days before the start of DSS administration. For the examination of the therapeutic effect of TN-3 on the colitis, the administration of TN-3 (10mg/day in 0.3ml PBS) started simultaneously with starting of DSS. The mice were euthanized at day12 under diethyl ether anesthesia by quick cervical distortion to minimize animal suffering. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. This study protocol was approved by
the Animal Care and Use Committee of Shiga University of Medical Science (Otsu, Japan) (Permit number:2013-9-8).

Preparation of *E. durans* TN-3

*E. durans* strain TN-3 was isolated from deep-sea water in Toyama bay. TN-3 was cultures for 18h at 30°C in a broth medium containing 2.46% (w/v) glucose, 1.4% (w/v) yeast extract, 0.77% (w/v) peptone and 4.39% (w/v) K₂HPO₄. After cultivation, the cells were collected by centrifugation and washed with distilled water. Heat-killed TN-3 was treated by autoclave for 10 min at 110°C and then lyophilized.

Assessment of inflammation in DSS-induced colitis

Mucosal inflammation was assessed using the disease activity index (DAI) described previously [18]. Histologic evaluations were performed in a blinded fashion using a validated scoring system [19].

Real-time polymerase chain reaction (real-time PCR)

The mRNA expression in the samples was assessed by real-time-polymerase chain reaction (PCR) analyses using a Light Cycler 480 system (Roche Applied Science, Tokyo, Japan) and SYBR Premix Ex Taq II (TAKARA, Otsu, Japan). The data were normalized versus β-actin mRNA. The oligonucleotide primers used in this study are shown in Table 1.

Immunohistochemistry

Immunohistochemical analyses were performed according to a method described in our previous report [20]. Briefly, rabbit anti-IL-1β (clone H-153; Santa Cruz biotechnology Inc., Dallas, TX), goat anti-IL-6 (clone H-19; Santa Cruz Biotechnology Inc.), rat-anti-IFN-γ (clone XMG1.2; BioLegend, San Diego, CA), rabbit anti-IL-17A (clone H-132; Santa Cruz Biotechnology Inc.), and goat anti-IL-10 antibody (clone M-18; Santa Cruz Biotechnology Inc.) were used as the primary antibodies. After incubation with the primary antibodies, the sections were treated with HRP (horseradish peroxidase)-labeled anti-rabbit IgG, anti-goat IgG, or anti-rat IgG antibodies. Diaminobenzidine was used as a substrate for color development.

Table 1. PCR primers used in this study.

| Gene   | Accession number | Primers  |
|--------|-----------------|----------|
| IL-1β  | NM_008361       | sense    |
|        |                 | anti-sense |
| IL-6   | NM_031168       | sense    |
|        |                 | anti-sense |
| IL-10  | NM_010548       | sense    |
|        |                 | anti-sense |
| IL-17A | NM_010552       | sense    |
|        |                 | anti-sense |
| IFN-γ  | NM_008337       | sense    |
|        |                 | anti-sense |
| β-actin| NM_007393       | sense    |
|        |                 | anti-sense |

doi:10.1371/journal.pone.0159705.t001
Cell isolation and flow cytometry

Mononuclear cells were isolated from the lamina propria of the colon. The isolated cells were stained with PE-labeled anti-CD4 (clone RM4-4; eBioscience, San Diego, CA), Alexa Fluor 488-labeled anti-Foxp3 (clone MF-14; BioLegend), PECy7-labeled anti-F4/80 (clone BM8; eBioscience), and APC-labeled anti-Gr-1 antibody (clone RB6-8C5; eBioscience). The cells were analyzed using FACS Calibur (BD Biosciences, Franklin Lake, NJ) according to the methods described previously [21, 22].

High-performance liquid chromatography

High-performance liquid chromatography (HPLC) was carried out for the analysis of stool extracts as previously described [23]. HPLC was performed using an Agilent 1120 Compact LC system (Santa Clara, CA) and a COSMOSIL 4.6×150mm 5C18-AR-II column (nacalai tesque inc., Kyoto, Japan).

DNA extraction and terminal restriction fragment length polymorphism (T-RFLP) analysis

DNA samples from feces were isolated using the method described previously [24]. The final concentration of DNA sample was adjusted to 10 ng/μl. T-RFLP analysis of the gut microbiota was performed according to the method described previously [24]. The T-RF fragments were divided into 30 operational taxonomic units (OTUs) as described by Nagashima et al. [25]. The prediction of bacteria was performed according to the BsaI-digested T-RFLP database [25]. The diversity among the different samples was compared by the Shannon diversity index (SDI) [26, 27].

Statistical analysis

The statistical significance of the differences was determined by Mann-Whitney U test. Differences resulting in P values less than 0.05 were considered to be statistically significant.

Results

Effects of TN-3 on the development of DSS colitis

To evaluate the preventive effects of TN-3 on the development of DSS colitis, we treated mice with TN-3 for 7 days prior to the start of DSS administration. As shown in Fig 1A, body weight (BW) was significantly lower in the DSS mice as compared to the DSS plus TN-3 mice. The disease activity index (DAI) was significantly higher in the DSS mice than the DSS plus TN-3 mice (Fig 1B). Furthermore, the histological inflammatory score was significantly lower in the DSS plus TN-3 mice than in the DSS mice (Fig 2A and 2B). The infiltration of immune cells also reflects the severity of colitis. Flow cytometric analysis for CD4⁺ T cells, F4/80⁺ macrophages, and Gr-1⁺ neutrophils in the colonic lamina propria was performed. As shown in Fig 3A and 3B, the infiltration of CD4⁺ T cells and Gr-1⁺ neutrophils was significantly suppressed in the DSS plus TN-3 mice as compared to the DSS mice. The infiltration of F4/80⁺ macrophages in the DSS plus TN-3 was also suppressed as compared to the DSS mice, but there was no significant difference between two groups.

We also examined the therapeutic effect of TN-3 on the development of colitis. We started the treatment of TN-3 at the same time of the starting of DSS administration. As shown in S1 Fig, BW in the TN-3 plus DSS mice was as low as the DSS mice, and the DAI in TN-3 plus DSS mice as high as the DSS mice. These results suggested that TN-3 has the preventive effect, but not the therapeutic effect, on the development of colitis.
Effect of TN-3 on the mucosal mRNA expression of proinflammatory cytokines

The mRNA expression of cytokines in the colonic mucosa was analyzed using real-time PCR. As shown in Fig 4, the mRNA expression of IL-1β, IL-6, IL-17A and IFN-γ decreased significantly in the DSS plus TN-3 mice as compared to the DSS mice. The mRNA expression of TNF-α decreased in the DSS plus TN-3 mice as compared to the DSS mice, but there was no significance between these two groups. Interestingly, we found that the mRNA expression of IL-10, which is an anti-inflammatory cytokine, was significantly elevated in the DSS plus TN-3 mice as compared to the DSS mice. As shown in Fig 5, we also confirmed that the expression of IL-1β, IL-6, IL-17A, and IFN-γ decreased, and the expression of IL-10 increased in the colon tissues of the DSS plus TN-3 mice as compared to the DSS mice using immunohistochemistry. Thus, TN-3 significantly suppressed the expression of proinflammatory cytokines and enhanced IL-10 expression.

Effect of TN-3 on the induction of regulatory T cells (Treg cells)

To explore the mechanism underlying the effects of TN-3 on DSS colitis, we focused on Treg cells in the colonic mucosa. Treg cells are known as a major source of IL-10 and have anti-inflammatory effects on the development of colitis [28]. As shown in Fig 6A, Treg cells were detected as CD4^+^Foxp3^+^ double positive cells by flow cytometry. As shown in Fig 6B, the
proportion of Treg cells was not altered in the TN-3 or DSS mice as compared to the control mice, but significantly increased in the DSS plus TN-3 mice as compared to the DSS mice. These results suggest that the preventive effects of TN-3 on the development of DSS colitis might be mediated by the induction of Treg cells in the colonic mucosa.

**Effect of TN-3 on fecal short-chain fatty acid (SCFA) levels**

Short-chain fatty acids (SCFAs), such as butyrate, acetate and propionate, are generated by the fermentation of dietary fibers by anaerobic bacteria [29]. Recent studies reported that butyrate plays a crucial role in the induction of mucosal Treg cells [30, 31]. Therefore, we examined the effects of TN-3 on fecal SCFA levels. As shown in Fig 7, fecal butyrate and acetate levels significantly increased in the TN-3 mice as compared to the control mice. Fecal butyrate levels significantly decreased in the DSS mice as compared to the control mice, but increased significantly in the DSS plus TN-3 mice as compared to the DSS mice. On the other hand, acetate levels significantly increased in the TN-3 mice as compared to the control mice, but there was no difference between the DSS and the DSS plus TN-3 mice. We also measured the fecal propionate levels, but there was no significant difference between the TN-3 mice and the control mice, and between the DSS and the DSS plus TN-3.
Effects of TN-3 on the fecal microbial composition and diversity

We investigated the effects of TN-3 on the fecal microbial structure using the T-RFLP method. The prediction of bacteria was performed according to the BsiI-digested T-RFLP database [25].
As shown in Fig 8A and Table 2, the results of bacteria prediction by T-RFLP analysis showed that proportion of *Bacteroides* significantly decreased and that of *Clostridium* cluster XI significantly increased in the TN-3 mice as compared to the control mice. In the DSS mice, the proportion of *Bacteroides* and *Clostridium* subcluster XIVa significantly increased and that of *Clostridium* cluster XI significantly decreased as compared to the control mice. However, there was no significant difference in the fecal microbial structure between the DSS mice and the DSS plus TN-3 mice. Next, we calculated the Shannon diversity index in each group. As shown in Fig 8B, the microbial diversity increased significantly in the TN-3 mice but decreased significantly in the DSS mice as compared to the control mice. However, the microbial diversity significantly increased in the DSS plus TN-3 mice as compared to the DSS mice. There was no significant difference in the diversity of microbial community between the control mice and the DSS plus TN-3 mice.
IBD is heterogeneous diseases characterized by overly aggressive immune responses to a subset of gut bacteria in genetically susceptible individual [3]. The relative imbalance of aggressive and protective bacterial species, termed dysbiosis, has been reported to be one of critical factors involved in the pathogenesis of IBD [4, 32]. Recent studies suggest that the therapeutic approaches targeting the gut microbiota, such as the use of probiotics, prebiotics and synbiotics, may improve the clinical outcome of patients with IBD [3]. The efficacy of probiotics for IBD has been reported. There are some large clinical trials of probiotics in IBD, especially in UC, in the setting of remission and maintenance of remission. A clinical trial demonstrated that *E. coli* Nissle 1917 was similar in efficacy to mesalamine for maintaining UC in remission [33]. *E. coli* Nissle 1917 is considered an effective alternative to mesalazine for maintenance of remission in UC. Two clinical trials suggested that the use of multistrain probiotic VSL#3 for

![Fig 5. The expression of cytokines in the colon tissue. The expression of IL-1\(^\beta\), IL-6, IL-17A, IFN-\(\gamma\), and IL-10 in the colon tissues was examined using immunohistochemistry. Control staining was also presented. The data are representative of four independent experiments. Magnification x 100.](https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0159705)
moderate active UC was able to improve the remission rate and the clinical response rate [34, 35].

Most probiotic microorganisms are classified as lactic acid bacteria, such as *Lactobacillus* spp., *Bifidobacterium* spp. and *Enterococcus* spp.[9] *Enterococcus* spp. strain TN-3 was isolated from deep seawater in Toyama bay in Japan [12]. TN-3 causes liquefaction of gelatin, fermentation of litmus milk, and possesses β-galactosidase activity. TN-3 has high homology to *E. durans* with respect to its 16S rDNA nucleotide sequences [12]. Irit Raz et al. suggested that *E. durans* has the immunoprotective and anti-inflammatory effect in DSS-induced colitis. They isolated *E. durans*, which was able to produce butyrate, from human feces, and demonstrated that *E. durans* suppressed DSS-induced colitis by colonizing in the colon and supplying adequate butyrate to colonocytes [36]. However, the mechanisms of suppressive effect of *E. durans* remained unclear. In the current study, we showed that *E. durans* TN-3 suppresses the development of DSS colitis via the induction of mucosal Treg cells. This was accompanied by the restoration of the fecal microbial diversity and an increase in fecal butyrate levels.

Fig 6. Proportion of Treg cells in the lamina propria of the colon. (A) Flow cytometric analysis for Foxp3⁺CD4⁺ T reg cells in the colonic lamina propria. Data are representative of five independent experiments. (B) Proportion of Foxp3⁺CD4⁺ T reg cells. Data are expressed as means ± SD of five different samples. *P < 0.05, **P < 0.01, n.s.; not significant.

doi:10.1371/journal.pone.0159705.g006

*Enterococcus* durans TN-3 Suppresses Murine Colitis
A number of studies have demonstrated that IL-10 is a key immunomodulatory factor that inhibits the release of proinflammatory cytokines by immune and inflammatory cells [37, 38], and that Treg cells are considered as a major source of IL-10 in the intestinal mucosa [39, 40]. In this study, we found that both IL-10 mRNA expression and the proportion of Treg cells increased significantly in the DSS plus TN-3 mice as compared to the DSS mice. These results suggest that \textit{E. durans} TN-3 might induce Treg cells and stimulate IL-10 production, leading to the suppression of the development of DSS colitis.

Recent studies demonstrated that butyrate, generated by the fermentation of dietary fiber by anaerobes, plays a crucial role in the induction of Treg cells in the mucosa [32, 41]. Other studies have demonstrated that butyrate has an anti-inflammatory property by suppressing the activation of transcription factor NF-\(\kappa\)B, which is a central transcription factor mediating various inflammatory responses [42, 43]. Based on these findings, we hypothesized that TN-3 modulated the gut microbial community and stimulated butyrate production, leading to the induction of Treg cells. As we expected, the diversity of fecal microbial community was restored in the TN-3 plus DSS mice as compared to the DSS mice, and fecal butyrate levels were significantly elevated in the TN-3 plus DSS mice as compared to the DSS mice. These findings suggest that \textit{E. durans} TN-3 modulated the gut microbial community and stimulated butyrate production. Subsequently, Treg cells were induced in response to increased butyrate generation, leading to the suppression of the development of DSS colitis. Additionally, butyrate
Fig 8. Effects of *E. durans* TN-3 on the gut microbial community and its diversity. (A) Changes in the fecal bacteria in DSS- and TN-3-treated mice. The value indicates the percentage of the predicted bacteria. (B) The Shannon Diversity Index (SDI) in comparison of the fecal bacterial diversity between groups. SDI was calculated from the BslI-digested terminal restriction fragment patterns. Data are expressed as means ± SD of five different samples.

doi:10.1371/journal.pone.0159705.g008

Table 2. Comparison of predicted bacteria.

| Predicted bacteria          | control      | TN-3         | DSS+PBS      | DSS+TN-3     |
|---------------------------|--------------|--------------|--------------|--------------|
| *Bifidobacteriales*        | 0.2 ± 0.4    | 0.0          | 0.0          | 0.0          |
| *Lactobacillales*          | 8.1 ± 2.6    | 9.4 ± 6.1    | 5.0 ± 3.9    | 5.6 ± 2.2    |
| *Bacteroides*              | 43.6 ± 9.4²  | 30.6 ± 6.5ᵇ  | 59.0 ± 8.3ᶜ  | 52.5 ± 6.0ᵃᵇᶜ |
| *Prevotella*               | 3.0 ± 1.2    | 4.6 ± 1.2    | 4.0 ± 3.3    | 7.3 ± 4.3    |
| *Clostridium*              | 18.0 ± 3.5ᵃ  | 25.4 ± 1.6ᵇ  | 25.9 ± 6.6ᵃᵇ | 27.0 ± 3.2ᵇ |
| *Clostridium cluster IV*   | 1.0 ± 0.61   | 0.9 ± 0.33   | 1.1 ± 0.33   | 0.8 ± 0.35   |
| *Clostridium subcluster XIVa* | 7.3 ± 2.06ᵃ  | 11.8 ± 3.9ᵃ  | 22.5 ± 6.1ᵇ  | 21.5 ± 3.4ᵇ  |
| *Clostridium cluster XI*   | 6.3 ± 1.4ᵃ   | 10.9 ± 2.7ᵇ  | 1.3 ± 0.9ᶜ   | 2.5 ± 1.3ᶜ   |
| *Clostridium cluster XVIII* | 3.4 ± 1.5ᵃ   | 1.8 ± 1.1ᵃᵇ  | 1.0 ± 0.27ᵇ  | 2.2 ± 1.1ᵃᵇ  |
| Others                     | 27.2 ± 8.1ᵃ  | 30.1 ± 6.3ᵃᵇ | 6.1 ± 3.6ᵇ  | 11.0 ± 11.0ᵇ |

Each value indicates the percentage of individual predicted bacteria. Values were expressed as mean ± SD. Values not sharing a letter are significantly different.

doi:10.1371/journal.pone.0159705.t002
might directly blocked the development of DSS colitis via its inhibitory action on NF-κB activation.

There are some limitations in this study. First, we did not clearly demonstrate how TN-3 modulates the gut microbiota. Second, there is no direct evidence that the increase expression of IL-10 produced by Treg cells is critical for the improvement of DSS-induced colitis. In the future, further examinations are needed to clarify these limitations.

In conclusion, the preventive administration of *E. durans* TN-3 suppressed the development of DSS colitis via the induction of IL-10 producing Treg cells by restoring of the diversity of gut microbiota. These findings suggest that *E. durans* TN-3 is a new probiotic candidate for the treatment of IBD.

**Supporting Information**

**S1 Fig. The effect of therapeutic treatment of TN-3 on the development of colitis.** BALB/cAJcl mice were orally inoculated with *E. durans* TN-3 (10mg/day) at the same time with the start of 4% DSS treatment. The mice were sacrificed at day12 for the experiments. (A) Changes in body weight. (B) Disease activity index on day 12. Data are expressed as means ± SD (n = 4 mice/group). n.s.; not significant.

(TIF)

**Acknowledgments**

Authors appreciate technical supports of TechnoSuruga Laboratory Co., Ltd. (Sizuoka, Japan) and would like to express thanks. Authors thank Dr. Masahiro Kawahara for his useful advice and technical help. This study was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (15K08967, 15K19322), a grant for the Intractable Diseases from the Ministry of Health, Labor and Welfare of Japan (067), and a grant from Smoking Research Foundation (1828).

**Author Contributions**

Conceived and designed the experiments: TK AN Performed the experiments: TK AN. Analyzed the data: TK AN. Contributed reagents/materials/analysis tools: TK AN MO HI TS OI SB MS AA. Wrote the paper: TK AA AN.

**References**

1. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007; 448(7152):427–34. PMID: 17653185
2. Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol. 2010; 28:573–621. doi: 10.1146/annurev-immunol-030409-101225 PMID: 20192811
3. Sheehan D, Moran C, Shanahan F. The microbiota in inflammatory bowel disease. J Gastroenterol. 2015; 50(5):495–507. doi: 10.1007/s00535-015-1064-1 PMID: 25808229
4. Goldsmith JR, Sartor RB. The role of diet on intestinal microbiota metabolism: downstream impacts on host immune function and health, and therapeutic implications. J Gastroenterol. 2014; 49(5):785–98. doi: 10.1007/s00535-014-0953-z PMID: 24652102
5. Mizoguchi A, Mizoguchi E. Inflammatory bowel disease, past, present and future: lessons from animal models. Journal of gastroenterology. 2008; 43(1):1–17. doi: 10.1007/s00535-007-2111-3 PMID: 18297430
6. Mayer L. Evolving paradigms in the pathogenesis of IBD. Journal of gastroenterology. 2010; 45(1):9–16. doi: 10.1007/s00535-009-0138-3 PMID: 19960355
7. Sands BE. Inflammatory bowel disease: past, present, and future. Journal of gastroenterology. 2007; 42(1):16–25. PMID: 17322989
8. Hill C, Guaner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014; 11(8):506–14. doi: 10.1038/nrgastro.2014.66 PMID: 24912386

9. Klein G, Pack A, Bonaparte C, Reuter G. Taxonomy and physiology of probiotic lactic acid bacteria. International journal of food microbiology. 1998; 41(2):103–25. PMID: 9704860

10. Nami Y, Abdullah N, Haghshenas B, Radiah D, Rosli R, Khosroushahi AY. Probiotic assessment of Enterococcus durans 6HL and Lactococcus lactis 2HL isolated from vaginal microflora. J Med Microbiol. 2014; 63(Pt 8):1044–51. doi: 10.1099/jmm.0.074161-0 PMID: 24913559

11. Avram-Hananel L, Stock J, Parlesak A, Bode C, Schwartz B. E durans strain M4-5 isolated from human colonic flora attenuates intestinal inflammation. Dis Colon Rectum. 2010; 53(12):1676–86. doi: 10.1007/DCR.0b013e3181f4b148 PMID: 21178864

12. Kondoh M, Hayashi A, Okamori M, Motonaga C, Enomoto T, Cheng L, et al. Effects of Enterococcus sp. isolated from deep seawater on inhibition of allergic responses in mice. Br J Nutr. 2009; 102(1):3–7. doi: 10.1017/S0007114508158998 PMID: 19079946

13. Kawada M, Anhiro A, Mizoguchi E. Insights from advances in research of chemically induced experimental models of human inflammatory bowel disease. World J Gastroenterol. 2007; 13(42):5581–93. PMID: 17948932

14. Mizoguchi A. Animal models of inflammatory bowel disease. Prog Mol Biol Transl Sci. 2012; 105:263–320. doi: 10.1016/B978-0-12-394596-9.00009-3 PMID: 22137435

15. Chassaing B, Aitken JD, Malleshappa M, Vijay-Kumar M. Dextran sulfate sodium (DSS)-induced colitis regulates T helper 17-cell responses and colitis in mice. Gastroenterology. 2012; 142(4):865–74. doi: 10.1016/S0016-5085(12)00266-8 PMID: 22622350

16. Clapper ML, Cooper HS, Chang WC. Dextran sulfate sodium-induced colitis-associated neoplasia: a promising model for the development of chemopreventive interventions. Acta pharmacologica Sinica. 2007; 28(9):1450–9. PMID: 17723178

17. Okayasu I, Hatakeyama S, Yamada M, Okhusa T, Inagaki Y, Nakaya R. A novel method in the induction of reliable experimental acute and chronic ulcerative colitis in mice. Gastroenterology. 1990; 98(3):694–702. PMID: 1688816

18. Cooper HS, Murthy SN, Shah RS, Sedergran DJ. Clinicopathologic study of dextran sulfate sodium experimental murine colitis. Laboratory investigation; a journal of technical methods and pathology. 1993; 69(2):238–49. PMID: 8350599

19. Rath HC, Herfarth HH, Ikeda JS, Grenther WB, Hamm TE Jr., Balish E, et al. Normal luminal bacteria, especially Bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. The Journal of clinical investigation. 1996; 98(4):945–53. PMID: 8770866

20. Fujino S, Andoh A, Bamba S, Ogawa A, Hata K, Araki Y, et al. Increased expression of interleukin 17 in inflammatory bowel disease. Gut. 2003; 52(1):65–70. PMID: 12477762

21. Nishida A, Nagahama K, Imaeda H, Ogawa A, Lau CW, Kobayashi T, et al. Inducible colitis-associated glycome capable of stimulating the proliferation of memory CD4+ T cells. J Exp Med. 2012; 209(13):2383–94. doi: 10.1084/jem.20112631 PMID: 23209314

22. Nishida A, Lau CW, Zhang M, Andoh A, Shi HN, Mizoguchi E, et al. The membrane-bound mucin Muc1 regulates T helper 17-cell responses and colitis in mice. Gastroenterology. 2012; 142(4):865–74 e2. doi: 10.1053/j.gastro.2011.12.036 PMID: 22202458

23. Torii T, Kanemitsu K, Wada T, Itoh S, Kinugawa K, Hagiwara A. Measurement of short-chain fatty acids in human faeces using high-performance liquid chromatography: specimen stability. Annals of clinical biochemistry. 2010; 47(Pt 5):447–52. doi: 10.1258/acb.2010.010047 PMID: 20595408

24. Andoh A, Imaeda H, Aomatsu T, Inatomi O, Bamba S, Sasaki M, et al. Comparison of the fecal microbiota profiles between ulcerative colitis and Crohn's disease using terminal restriction fragment length polymorphism analysis. J Gastroenterol. 2011; 46(4):479–86. doi: 10.1007/s00535-010-0368-4 PMID: 21253779

25. Nagashima K, Hisada T, Sato M, Mochizuki J. Application of new primer-enzyme combinations to terminal restriction fragment length polymorphism profiling of bacterial populations in human feces. Appl Environ Microbiol. 2003; 69(2):1251–62. PMID: 12571054

26. Dean-Ross D, Mills AL. Bacterial Community Structure and Function along a Heavy Metal Gradient. Applied and environmental microbiology. 1989; 55(8):2002–9. PMID: 16347991

27. Hill TC, Walsh KA, Harris JA, Moffett BF. Using ecological diversity measures with bacterial communities. FEMS microbiology ecology. 2003; 43(1):1–11. doi: 10.1111/j.1574-6941.2003.tb01040.x PMID: 19719691
28. Zeng H, Chi H. Metabolic control of regulatory T cell development and function. Trends in immunology. 2015; 36(1):3–12. doi: 10.1016/j.it.2014.08.003 PMID: 25248463

29. Andoh A. Physiological Role of Gut Microbiota for Maintaining Human Health. Digestion. 2016; 93(3):176–81. doi: 10.1159/000444406 PMID: 26859303

30. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013; 500(7461):232–6. doi: 10.1038/nature12331 PMID: 23842501

31. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. Science. 2011; 331(6015):337–41. doi: 10.1126/science.1198469 PMID: 22105640

32. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature. 2013; 504(7480):451–5. doi: 10.1038/nature12726 PMID: 24226773

33. Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, Kascak M, et al. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. Gut. 2004; 53(11):1617–23. PMID:15479682

34. Tursi A, Brandimarte G, Papa A, Giglio A, Elisei W, Giorgetti GM, et al. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL#3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. Am J Gastroenterol. 2010; 105(10):2218–27. doi:10.1038/ajg.2010.218 PMID: 20517305

35. Sood A, Midha V, Makharia GK, Ahuja V, Singal D, Goswami P, et al. The probiotic preparation, VSL#3 induces remission in patients with mild-to-moderately active ulcerative colitis. Clin Gastroenterol Hepatol. 2009; 7(1):1202–9, 9 e1. doi: 10.1016/j.cgh.2009.07.016 PMID: 19631292

36. Raz I, Gollop N, Polak-Charcon S, Schwartz B. Isolation and characterisation of new putative probiotic bacteria from human colonic flora. Br J Nutr. 2007; 97(4):725–34. PMID: 17349085

37. Maynard CL, Harrington LE, Janowski KM, Oliver JR, Zindl CL, Rudensky AY, et al. Regulatory T cells expressing interleukin 10 develop from Foxp3+ and Foxp3- precursor cells in the absence of interleukin 10. Nat Immunol. 2007; 8(9):931–41. PMID: 17694059

38. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011; 474(7351):298–306. doi: 10.1038/nature10208 PMID: 21677746

39. Kindlund B, Sjoling A, Yakkala C, Adamsson J, Janson A, Hansson LE, et al. CD4 regulatory T cells in gastric cancer mucosa are proliferating and express high levels of IL-10 but little TGF-beta. Gastric Cancer. 2016.

40. Zheng SG, Wang JH, Gray JD, Soucier H, Horwitz DA. Natural and induced CD4+CD25+ cells educate CD4+CD25- cells to develop suppressive activity: the role of IL-2, TGF-beta, and IL-10. J Immunol. 2004; 172(9):5213–21. PMID: 15100259

41. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. Nature. 2013; 504(7480):446–50. doi: 10.1038/nature12721 PMID: 24226770

42. Inan MS, Rasoulpour RJ, Yin L, Hubbard AK, Rosenberg DW, Giardina C. The luminal short-chain fatty acid butyrate modulates NF-kappaB activity in a human colonic epithelial cell line. Gastroenterology. 2000; 118(4):724–34. PMID: 10734024

43. Yin L, Laevsky G, Giardina C. Butyrate suppression of colonocyte NF-kappa B activation and cellular proteasome activity. J Biol Chem. 2001; 276(48):44641–6. PMID: 11572859