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Goji Berry Modulates Gut Microbiota and Alleviates Colitis in IL-10-Deficient Mice

*Yifei Kang, Guan Yang, Shuming Zhang, Carolyn F. Ross, and Mei-Jun Zhu**

Scope: This study examines the beneficial effects of Goji berry against spontaneous colitis and its prebiotic role in IL-10-deficient mice.
Methods: IL-10-deficient mice are assigned to a standard rodent diet (control) or a control diet supplemented with Goji (1% of dry feed weight) for 10 weeks, at which point colonic tissues and fecal contents are collected.
Results: Goji supplementation decreases colonic pathobiological scores and mRNA expression of *Il17a* and *Tgfb1*, while it enhances *Muc1* expression and fecal IgA content. Illumina MiSeq sequencing reveals that Goji supplementation increases *Actinobacteria* phylum, resulting in a bloom of *Bifidobacteria* in gut microbiota. Additionally, dietary Goji promotes butyrate-producing bacteria including *Lachnospiraceae-Ruminococcaceae* family and Roseburia spp. under *Clostridium* cluster XIVa. Furthermore, butyrate-producers *Clostridium leptum* and its dominant constituent *Fecalibacterium prazusnitzii* are markedly increased in the Goji group. Moreover, the gene-encoding butyryl-coenzyme A CoA transferase, a key enzyme responsible for butyrate synthesis in butyrate-producing bacteria, is increased sixfold in the fecal samples of Goji group associated with increased fecal butyrate content.
Conclusion: Data collectively show that dietary Goji results in the blooming of *Bifidobacteria* and butyrate-producing bacteria. These bacteria may cross-feed each other, conferring preventative effects against colitis in IL-10-deficient mice.

1. Introduction

Inflammatory bowel diseases (IBD), which are rapidly growing epidemics, are distinguished as Crohn's disease (CD) and ulcerative colitis (UC) depending on the location and severity of the disease. IBD leads to increased incidence of colorectal cancer and is associated with increased morbidity and mortality rates.[1] IBD has a complex and obscure etiology driven by genetic predisposition,[2] dysregulated immune response, and environmental factors.[3] Despite the complexity of etiology, a wealth of evidence implicates gut microbiota as a pivotal factor in the IBD pathogenesis.[3,4]

IBD patients are associated with intestinal dysbiosis and reduced diversity of mucosal-associated/fecal microbiota.[5,6] The population of normal indigenous intestinal bacteria, including *Bacteroides*, *Eubacterium*, and *Lactobacillus*, was found to be reduced in IBD patients.[5] Butyrate-producing bacteria, such as *Roseburia* spp.,[7,8] *Clostridium leptum* group,[6] and its prominent component *Faecailbacterium prausnitzii*,[8,9] were also reduced in gut microbiota of IBD patients. Recurrently, low abundance of *Bifidobacteria* in gut microbiota was linked to IBD in children,[10] while increased sulfite-reducing bacterium *Bilophila wadsworthia*, due to high milk fat intake, was associated with the development of colitis in IL-10-deficient mice.[11] Conversely, *Bifidobacterium longum* 536 supplementation, in addition to standard treatment, reduced disease activity index scores in patients with mild to moderately active UC.[12]

Goji (*Lycium barbarum*) berry has long been consumed as a tonic food or a traditional nutraceutical supplement, and is known for its health-promoting effects including improvement of immune responses,[13] reduction in circulatory lipid content,[14] prevention of oxidative stress,[15] and protection of central nervous system.[16] Goji is also beneficial for intestinal health. *Lycium barbarum* polysaccharides (LBP) protects the small intestinal structure and contributes barrier function in the intestinal damage induced by ischemia reperfusion.[17] A milk-based Goji preparation exerted protective roles in 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis.[18] Dietary Goji ameliorated IBD symptoms in dextran sulfate sodium (DSS)-induced colitis mice accompanied with decreased neutrophil infiltration and suppressed inflammatory responses.[19]

Goji contains a range of phytochemicals, of which LBP is the most well-recognized and important functional constituent. LBP typically accounts for 5–8% of dried fruits,[20] which varies by the sources of Goji berry and extraction methods. It is predicted that a maximal yield of 23.3% of LBP can be achieved using optimized extraction technology.[21] Thus, Goji is expected to have prebiotic effects, which has not yet been assessed, nor the link of its prebiotic effects to its beneficial effects. Using IL-10-deficient mice, the most common used animal model for studying gut inflammation and etiology of IBD,[1,22] this study aimed to examine the preventative effects of Goji against gut inflammation and

Y. Kang, Dr. G. Yang, Dr. S. Zhang, Dr. C. F. Ross, Dr. M.-J. Zhu
School of Food Science
Washington State University
Pullman, WA 99164, USA
E-mail: meijun.zhu@wsu.edu

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colitis, and further explore its prebiotic/gut microbiota modulation effects.

2. Experimental Section

2.1. Animal Care and Experimental Design

Six-week-old male IL-10-deficient mice were randomized into two groups ($n = 7$ per group) assigned to a standard rodent diet (Con) or the same diet supplemented with 1% Goji for 10 weeks when mice were sacrificed for tissue collection. Table S1, Supporting Information shows the same composition for both diets. Detailed information about diets, animal experimental design, tissue, and fecal samples collection information is listed in the Supporting Information.

2.2. Histological and Immunohistochemical Examination

Paraffin-embedded colonic tissues were sectioned, subjected to haemotoxylin and eosin (H&E) staining, and imaging. Pathobiological examination was then conducted and scored in a blinded manner as previously described[23] with slight modifications. The macrophage immunohistochemical (IHC) staining with F4/80 primary antibody (Bio-Rad, Kidlington, OR) was carried out as described previously.[22] Details were described in Supporting Information.

2.3. Quantitative RT-PCR

Total RNA was extracted from colonic tissues using Trizol (Sigma) and purified with RNeasy Mini kit (Qiagen). cDNA synthesized with the iScript™ cDNA kit (Bio-Rad) was used for quantitative RT-PCR reaction with primers listed in Table S2, Supporting Information.

2.4. Fecal Sample IgA ELISA Analysis

Mouse fecal IgA was measured using a mouse IgA ELISA kit (eBioscience) per the manufacturer's instruction as described in Supporting Information.

2.5. Illumina 16S rRNA V4 Region Multiplex Amplicon Preparation, Sequencing, and Analysis

Colonic contents (fecal samples) were collected at necropsy, frozen in liquid nitrogen, and stored at –80 °C until analyzed. Fecal samples were powdered in liquid nitrogen and homogenized in lysis buffer (Qiagen, Valencia, CA) using a Polytron homogenizer (PT1200E Kinematic Inc., Bohemia, NY). Bacterial genomic DNA was extracted from fecal samples using a QIAamp DNA stool mini kit (Qiagen) according to the manufacturer's instructions.

Microbial sequencing was performed at Initiative for Bioinformatics and Evolutionary Studies (IBEST) Genomics Resources Core at the University of Idaho using Illumina MiSeq dual-barcoded two-step PCR amplicon sequencing. The details about fecal bacterial genomic DNA extraction, 16S rRNA V4 region multiplex amplicon preparation, sequencing, and analysis are described in Supporting Information.

2.6. qPCR of 16S rRNA for Specific Species or Genus

The abundance of specific bacterial groups was measured by quantitative PCR using fecal bacterial DNA and the primers listed in Table S3, Supporting Information.

2.7. SCFA Analysis

Fecal samples were prepared as previously described with modifications.[24] The SCFAs in feces were analyzed by a Hewlett Packard HP 6980 Series GC System equipped with a flame ionization detector and a fused-silica capillary ZB-WAX*plus* column (30 m × 0.25 mm id, 0.25 μm film thickness; Phenomenex Inc, USA). The external standard method was used to quantify their amounts. A temperature program with splitless injection was applied to separate SCFAs: The initial oven temperature was 80 °C, held for 5 min, increased to 140 °C by the rate of 15 °C min^{-1}, raised to 180 °C by 10 °C min^{-1} and held for 2 min, and then increased to 240 °C by 30 °C min^{-1} and held at 240 °C for 5 min. The data were expressed as the mean and SEM. An unpaired Student's *t*-test was carried out to analyze the significance of differences between Con and Goji groups.

2.8. Statistical Analysis

Data were analyzed as a complete randomized design using General Linear Model of Statistical Analysis System (2000). Data were expressed as mean ± SE. A significant difference was considered as p-value ≤ 0.05.

3. Results

3.1. Dietary Goji Reduces Colonic Inflammation and Pathobiological Scores in IL-10-Deficient Mice

Goji supplementation had no effect on body weight gain and feed intake in IL-10-deficient mice (**Figure 1**A,B). Histologically, Goji supplementation improved the colonic integrity of epithelial structure with reduced inflammation and restored overall crypt structure (Figure 1C), as shown by reduced pathological scores in both the proximal and distal colon (Figure 1C).

The IHC staining of F4/80, one of the best macrophage markers, indicated that the macrophage infiltration was decreased in the colon tissues of the Goji-supplemented mice (**Figure 2**A). Furthermore, the mRNA expression of *Muc1* in colonic tissues (Figure 2B) and the fecal IgA content (Figure 2C) were enhanced, while mRNA expression of inflammatory cytokine IL-17A and TGF-β (Figure 2D,E) was suppressed due to Goji supplementation.

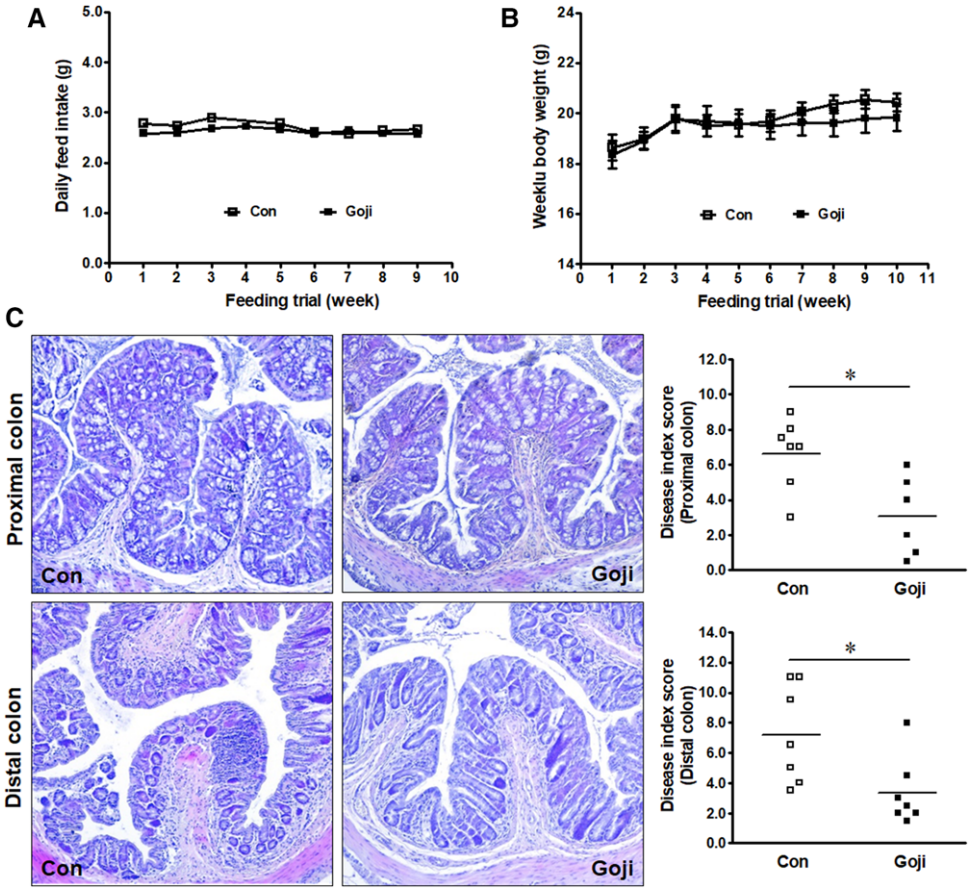

Figure 1. Histopathologic scores of proximal and distal colon in IL-10-deficient mice fed with Con (□) or Goji (■) supplemented diets. A) Feed intake; B) weekly body weight; C) histopathologic scores of proximal and distal colon. Representative images of hematoxylin and eosin stained colonic tissue (200×) are shown. Mean ± SEM, $n = 7$. * $p \leq 0.05$.

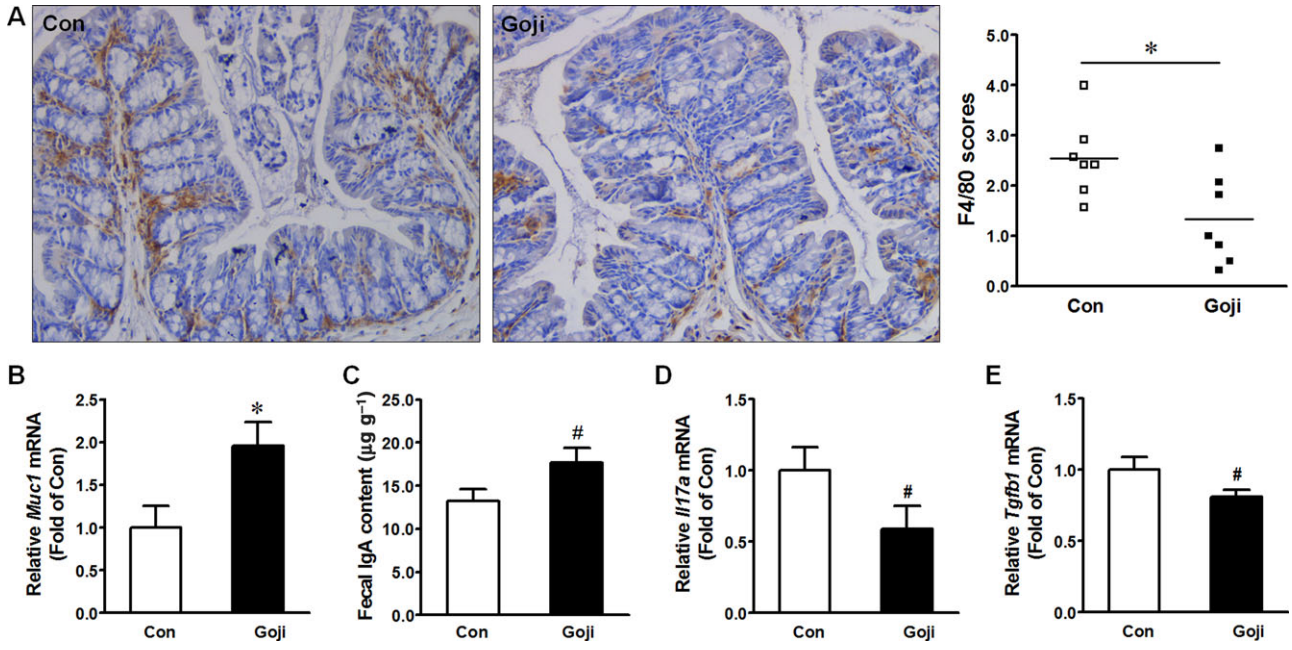

Figure 2. Dietary Goji berry reduced inflammation in colonic tissue of IL-10-deficient mice. A) Representative images and quantification score of F4/80 macrophage marker staining, magnification at 200×; B) mRNA expression of *Muc1*; C) fecal IgA content analyzed by ELISA; D) mRNA expression of *il17a*; E) mRNA expression of *Tgfb1*. Mean ± SEM, $n = 7$. $^{\#}p < 0.10$, $^{*}p \leq 0.05$.

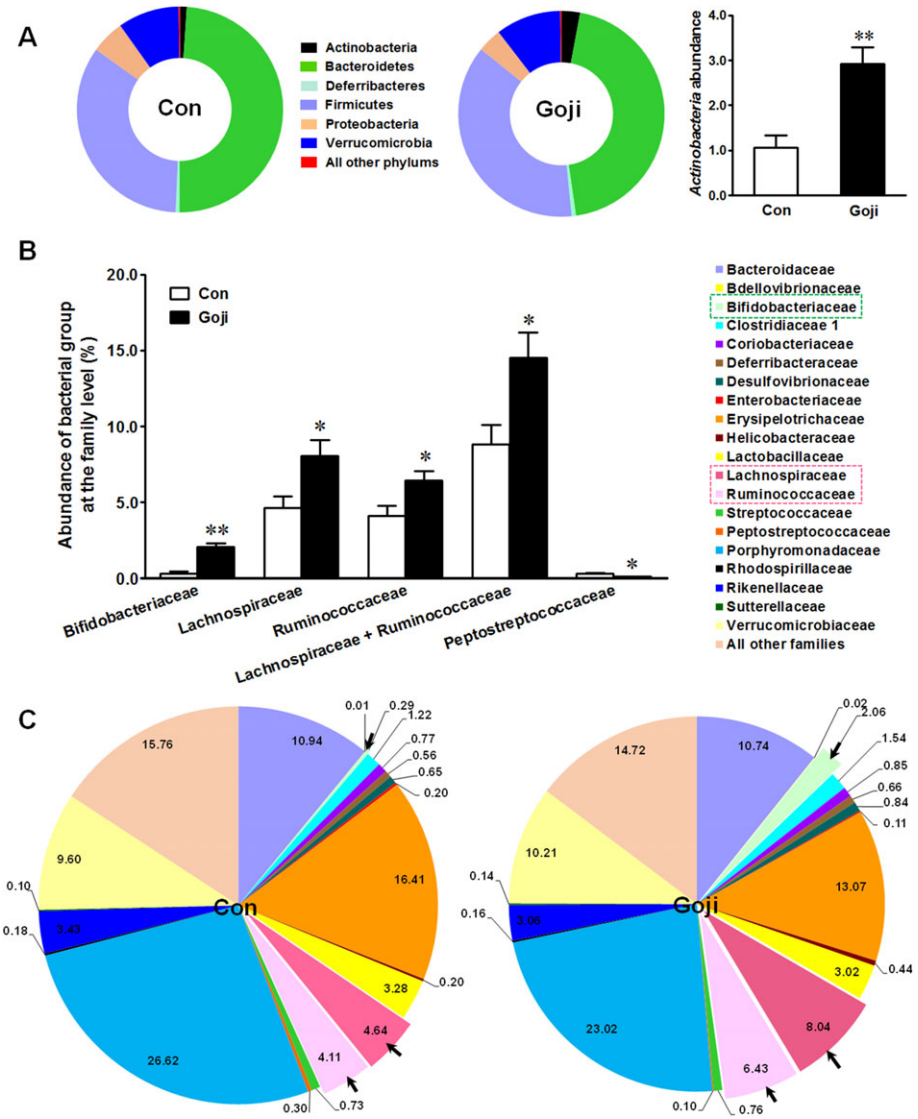

Figure 3. Gut microbiota composition and abundance in fecal samples of IL-10-deficient mice analyzed by Illumina MiSeq sequencing. A) Overall comparison of gut microbiota at phylum level; B) selected bacterial family; C) overall comparison of gut microbiota at family level between Con and Goji groups. Mean ± SEM, $n = 7$. *$p \leq 0.05$, **$p \leq 0.01$.

3.2. Goji Berry Shows Strong Prebiotic Effect in Gut of IL-10-Deficient Mice

Goji supplementation increased *Actinobacateria* in gut microbiota without changing other phyla (**Figure 3**A; Figure S1, Supporting Information) and the *Firmicutes* to *Bacteroidetes* ratio. The *Bifidobacteriaceae* bloomed due to Goji supplementation; its abundance in the Goji group was ~7 times of that in Con group (Figure 3B). Dietary Goji also increased *Lachnospiraceae* and *Ruminococcaceae* but decreased *Peptostreptococcaceae* abundance (Figure 3B,C). Strikingly, the *Lachnospiraceae–Ruminococcaceae* family accounted for 14.5 ± 1.7% in gut microbiota of Goji group compared to 8.8 ± 1.3 % in Con mice. At the genus level, the *Bifidobacterium* population of Goji was ~7 times of that of Con. Goji supplementation further enhanced *Clostridium* XIVb, *Clostridium* XVIII, *Pseudoflavonifractor*, *Sporobacter*, *Anaerotruncus*, *Butyriccicoccus*, and *Anaerosporobacter* (**Figure 4**A), but had no effect on *Bacteriodes*, *Akkemansia*, *Mucispirillum*, and *Desulfovirio* genus (Figure S2, Supporting Information). Quantitative PCR further confirmed a drastic increase in the population of *Bifidobacterium* in fecal samples of the Goji-supplemented mice (Figure 4B).

3.3. Goji Berry Enhances Butyrate-Producing Bacteria in Fecal Samples of IL-10-Deficient Mice

Aforementioned *Lachnospiraceae* and *Ruminococcaceae* families were classified in the *Clostridium* cluster XIVa group,[25] a major butyrate-producing group, which were drastically enriched in Goji group (Figure 3B). In addition, the abundance of *Clostridium* Cluster XIVa and *Roseburia* spp. within this group was increased

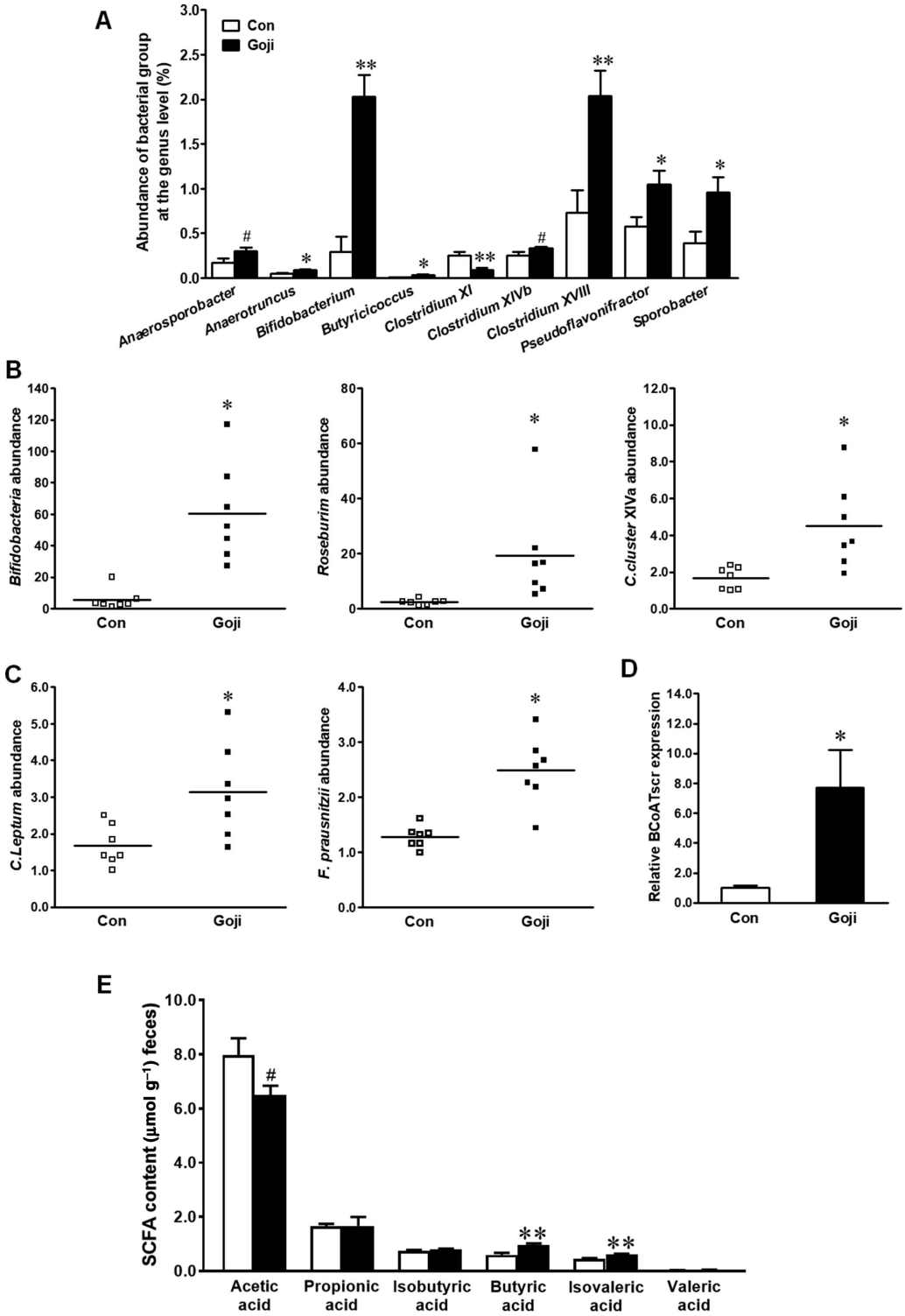

Figure 4. Selected bacterial group in fecal samples of IL-10-deficient mice fed with Con or Goji supplemented diets. A) Selected bacterial genus analyzed by Illumina MiSeq sequencing; B) quantification of *Bifidobacterium* spp., *Roseburia* spp., and *Clostridium XIVa* by quantitative PCR; C) the population of *Clostridium leptum* and *Faecailbacterium prausnitzii* measured by quantitative PCR; D) the abundance of butyryl-CoA CoA transferase (BCoATscr) in fecal of IL-10-deficient mice analyzed by quantitative PCR; E) quantification of short-chain fatty acids (SCFA) in fecal samples of IL-10-deficient mice by GC-FID. Mean ± SEM, $n = 7$. $^{\#}p \leq 0.10$, $^{*}p \leq 0.05$, $^{**}p \leq 0.01$.

(Figure 4B). Furthermore, the butyrate-producing *Clostridium leptum* group and its dominant constituent *F. prazusnitzii* were also markedly increased in fecal samples of Goji group (Figure 4C). Most butyrate-producing bacteria carry the gene encoding butyryl-Coenzyme A CoA transferase (BCoATscr), a key enzyme in butyrate formation.[26] Consistently, BCoATscr was increased more than sixfold in Goji group compared to Con (Figure 4D). In agreement, the butyrate and isovalerate content in feces of Goji group were higher than those in Con group (Figure 4E).

4. Disscussion

4.1. Goji Berry Has Strong Prebiotic Effects

Goji berry contains abundant LBP that is known for its health-beneficial effects.[20,21] LBP water-soluble extract consists of a complex mixture of extensively branched glycoconjugates with molecular weights between 10–2300 kD.[27] The water-soluble LBP is mainly pectic polysaccharides, but also contains glucan, xylan, arabinan, and arabionogalactan-protein, and the water-insoluble fraction contains pectin and hemicellulosic polysaccharides.[28] Thus, polysaccharides associated with Goji intake provide excellent prebiotics for promoting health beneficial microorganisms in gut microbiota.

Bifidobacteria is one of the best-known health beneficial microorganisms with protective effects against IBD.[12] The *Bifidobacteria* genome contains a large number of genes (>8% of the genome) associated with oligosaccharide catabolism, including more than 40 glycosyl hydrolases,[29] making it well adapted to utilize prebiotic polysaccharides. Accordingly, dietary Goji bloomed *Bifidobacteria* in fecal samples of IL-10-deficient mice, which were associated with the improved IBD colonic pathobiological scores. In agreement, oral administration of fructo-oligosaccharides enriched fecal *Bifidobacteria*, which was low in IBD patients,[10] and reduced disease activity in patients with CD.[30] Furthermore, the *Muc1* expression was enhanced in Goji group, in alignment with the role of probiotics as strong potentiators of mucin expression and secretion.[31] Goji supplementation also enriched the *Lachnospiraceae–Ruminococcaceae* family, which is reduced under chronic intestinal disorders, such as IBD.[9,32] Taken together, these data suggest strong prebiotic effects of Goji, which is plausibly responsible for improved epithelial structure in IL-10-deficient mice.

4.2. Goji Berry Promotes Butyrate-Producing Bacteria in Fecal Samples of IL-10-Deficient Mice

Butyrate, as a main energy source for colonic epithelial cells, plays an important role in intestinal homeostasis via multiple mechanisms.[33,34] The abundance of butyrate-producing bacteria is inversely correlated with IBD symptoms.[6–9] The majority of butyrate-producing strains belong to clusters XIVa and IV of *Clostridia*.[25,35] Consistent with improved IBD indices, the population of *Lachnospiraceae–Ruminococcaceae* and *Roseburia* spp. under *Clostridia* cluster XIVa were increased in IL-10-deficient mice supplemented with Goji. Similarly, fecal *F. prausnitzii*, the most prominent butyrate-producer, which is decreased in IBD patients,[8,9] was increased in Goji group. These results correlated with the suppressed IL-17A production, and alleviated inflammation and colitis symptoms. In alignment with above changes, oral administration of the culture supernatant of *F. prausnitzii*[36] or butyrate[37] suppressed IL-17 production and ameliorated TNBS-induced-colitic-lesion in rats.

The BCoATscr gene, representing a major butyrate formation pathway and existing in butyrate-producing bacteria, is a good indicator of the overall abundance of butyrate-producing bacteria in gut microbiota.[26,38] Consistently, dietary Goji resulted in a profound increase of BCoATscr in gut microbiota of IL-10-deficient mice. In addition, *Bifidobacteria* and butyrate-producing bacteria could cross-feed each other to form a synergistic relationship in producing butyrate.[39] In aggregate, the enhanced butyrate-producing bacteria in addition to *Bifidobacteria* due to Goji supplementation likely exert beneficial effects against colitis in IL-10-deficient mice.

4.3. Dietary Goji Berry Suppresses Inflammation in Intestine of IL-10-Deficient Mice

Chronic inflammation is indispensable for the etiology of IBD. Due to the deficiency of an important anti-inflammatory cytokine, IL-10-deficient mice develop chronic inflammation, which leads to spontaneous colitis.[40] Intestinal macrophages play an important role in induction of innate immune responses and maintenance of intestinal homeostasis.[41] IL-17 is the signature effector cytokine of Th17 cells and is elevated in IBD patients.[42] Consistently, Goji supplementation decreased colonic IL-17A expression and macrophage infiltration in IL-10-deficient mice. Similarly, dietary Goji alleviated disease index activity and suppressed neutrophil infiltration and inflammation in DSS-induced-colitis.[19] A milk-based Goji preparation conferred protective roles in TNBS-induced-colitis mice through its anti-inflammatory effects.[18]

The TGF-β signaling has an important role in suppression of gut inflammation.[43] Acute inflammation induces TGF-β signaling,[44] which promotes the switch from Th17 cells to regulatory T (Treg) cells, transiting from proinflammatory to anti-inflammatory state during wound healing.[45] However, chronic inflammation in IBD patients and IL-10-deficient mice renders the anti-inflammatory transition impossible. In these cases, the severity of chronic inflammation is correlated with sustained TGF-β signaling,[43] consistent with elevated TGF-β expression in inflamed colonic biopsy samples from UC or CD patients.[46] In agreement, correlated with suppressed inflammation and increased *Bifidobacteria*, TGF-β expression was decreased in Goji supplemented IL-10-deficient mice. On the other hand, in acute colitis induced by chemicals, *Bifidobacteria* enhanced TGF-β production,[47] which promotes anti-inflammatory transition and wound healing in the cecum of HLA-B27 transgenic rats,[48] and increases peripheral Treg cells.[49]

IgA is the major immunoglobulin produced in the intestinal mucosa. Fecal total IgA titer was lower in UC patients than that in a healthy control.[50] Consistently, fecal IgA secretion was enhanced in Goji group, further demonstrating the improvement

of gut mucosal immune response, given that the mucosal defense of healthy gut is primarily through IgA secretion.[51] In agreement with our results, supplementation of galactooligosaccharides increases both *Bifidobacteria* abundance and fecal IgA level.[52]

Though the exact mechanisms leading to the anti-inflammatory effects of dietary Goji berry remain to be established, the elevation of butyrate might have an important role.[53] As a major gut microbiota metabolite, butyrate is known to have strong anti-inflammatory effects.[53–55] Butyrate inhibits inflammatory signaling in immune cells,[53] facilitates epithelial differentiation, and inhibits proliferation,[33] as well as the assembly of tight junction complexes to strengthen epithelial barrier.[56] As a result, butyrate reduces bacterial translocation across gut epithelium[57] and thus suppresses inflammation.

In conclusion, Goji supplementation has strong prebiotic effects as indicated by the bloom of *Bifidobacteria* and butyrate-producing bacteria in IL-10-deficient mice. Associated with its prebiotic effects, dietary Goji ameliorates colonic inflammation and pathobiology related to IBD. Data collectively suggest that dietary Goji improves gut health and provides an alternative therapeutic measure for IBD patients.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

Bifidobacteria, butyrate-producing bacteria, Goji, gut microbiota, IBD, interleukin-10

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[1] D. J. Berg, N. Davidson, R. Kuhn, W. Muller, S. Menon, G. Holland, L. Thompson-Snipes, M. W. Leach, D. Rennick, *J. Clin. Invest.* **1996**, *98*, 1010.
[2] Y. Ogura, D. K. Bonen, N. Inohara, D. L. Nicolae, F. F. Chen, R. Ramos, H. Britton, T. Moran, R. Karaliuskas, R. H. Duerr, J. P. Achkar, S. R. Brant, T. M. Bayless, B. S. Kirschner, S. B. Hanauer, G. Nunez, J. H. Cho, *Nature* **2001**, *411*, 603.
[3] J. Miyoshi, E. B. Chang, *Transl. Res.* **2017**, *179*, 38.
[4] J. Ni, G. D. Wu, L. Albenberg, V. T. Tomov, *Nat. Rev. Gastroenterol. Hepatol.* **2017**, *14*, 573.
[5] S. J. Ott, M. Musfeldt, D. F. Wenderoth, J. Hampe, O. Brant, U. R. Folsch, K. N. Timmis, S. Schreiber, *Gut* **2004**, *53*, 685.
[6] C. Manichanh, L. Rigottier-Gois, E. Bonnaud, K. Gloux, E. Pelletier, L. Frangeul, R. Nalin, C. Jarrin, P. Chardon, P. Marteau, J. Roca, J. Dore, *Gut* **2006**, *55*, 205.
[7] X. C. Morgan, T. L. Tickle, H. Sokol, D. Gevers, K. L. Devaney, D. V. Ward, J. A. Reyes, S. A. Shah, N. LeLeiko, S. B. Snapper, A. Bousvaros, J. Korzenik, B. E. Sands, R. J. Xavier, C. Huttenhower, *Genome Biol.* **2012**, *13*, R79.
[8] D. Gevers, S. Kugathasan, L. A. Denson, Y. Vazquez-Baeza, W. Van Treuren, B. Ren, E. Schwager, D. Knights, S. J. Song, M. Yassour, X. C. Morgan, A. D. Kostic, C. Luo, A. Gonzalez, D. McDonald, Y. Haberman, T. Walters, S. Baker, J. Rosh, M. Stephens, M. Heyman, J. Markowitz, R. Baldassano, A. Griffiths, F. Sylvester, D. Mack, S. Kim, W. Crandall, J. Hyams, C. Huttenhower, et al. *Cell Host Microbe* **2014**, *15*, 382.
[9] H. Sokol, B. Pigneur, L. Watterlot, O. Lakhdari, L. G. Bermudez-Humaran, J. J. Gratadoux, S. Blugeon, C. Bridonneau, J. P. Furet, G. Corthier, C. Grangette, N. Vasquez, P. Pochart, G. Trugnan, G. Thomas, H. M. Blottiere, J. Dore, P. Marteau, P. Seksik, P. Langella, *Proc. Natl. Acad. Sci.* **2008**, *105*, 16731.
[10] J. Maukonen, K. L. Kolho, M. Paasela, J. Honkanen, P. Klemetti, O. Vaarala, M. Saarela, *J Crohns Colitis* **2015**, *9*, 1088.
[11] S. Devkota, Y. Wang, M. W. Musch, V. Leone, H. Fehlner-Peach, A. Nadimpalli, D. A. Antonopoulos, B. Jabri, E. B. Chang, *Nature* **2012**, *487*, 104.
[12] H. Tamaki, H. Nakase, S. Inoue, C. Kawanami, T. Itani, M. Ohana, T. Kusaka, S. Uose, H. Hisatsune, M. Tojo, T. Noda, S. Arasawa, M. Izuta, A. Kubo, C. Ogawa, T. Matsunaka, M. Shibatouge, *Dig. Endosc.* **2016**, *28*, 67.
[13] Z. H. Ren, L. X. Na, Y. M. Xu, M. Rozati, J. P. Wang, J. G. Xu, C. H. Sun, K. Vidal, D. Y. Wu, S. N. Meydani, *J. Nutr.* **2012**, *142*, 1596.
[14] M. Ma, G. H. Liu, Z. H. Yu, G. Chen, X. Zhang, *Food Chem.* **2009**, *113*, 872.
[15] Y. Yang, W. Li, Y. Li, Q. Wang, L. Gao, J. Zhao, *Oxid. Med. Cell Longev.* **2014**, *2014*, 145641.
[16] Y. S. Ho, M. S. Yu, S. Y. Yik, K. F. So, W. H. Yuen, R. C. Chang, *Cellular and Molecular Neurobiology* **2009**, *29*, 1233.
[17] X. Yang, H. Bai, W. Cai, J. Li, Q. Zhou, Y. Wang, J. Han, X. Zhu, M. Dong, D. Hu, *Chem Biol. Interact.* **2013**, *204*, 166.
[18] D. Philippe, V. Brahmbhatt, F. Foata, Y. Saudan, P. Serrant, S. Blum, J. Benyacoub, K. Vidal, *World J. Gastroenterol.* **2012**, *18*, 5351.
[19] Y. Kang, Y. Xue, M. Du, M. J. Zhu, *J. Nutr. Biochem.* **2017**, *40*, 70.
[20] J. Cheng, Z. W. Zhou, H. P. Sheng, L. J. He, X. W. Fan, Z. X. He, T. Sun, X. Zhang, R. J. Zhao, L. Gu, C. Cao, S. F. Zhou, *Drug Des. Devel. Ther.* **2015**, *9*, 33.
[21] G. H. Yin, Y. L. Dang, *Carbohydr. Polym.* **2008**, *74*, 603.
[22] G. Yang, Y. Xue, H. Zhang, M. Du, M. J. Zhu, *Br. J. Nutr.* **2015**, *114*, 15.
[23] A. Burich, R. Hershberg, K. Waggie, W. Zeng, T. Brabb, G. Westrich, J. L. Viney, L. Maggio-Price, *Am. J. Physiol. Gastrointest. Liver Physiol.* **2001**, *281*, G764.
[24] G. Zhao, M. Nyman, J. Jönsson, *Biomed. Chromatogr.* **2006**, *20*, 674.
[25] M. D. Collins, P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, J. A. Farrow, *Int. J. Syst. Bacteriol.* **1994**, *44*, 812.

[26] C. Charrier, G. J. Duncan, M. D. Reid, G. J. Rucklidge, D. Henderson, P. Young, V. J. Russell, R. I. Aminov, H. J. Flint, P. Louis, *Microbiology* **2006**, *152*, 179.
[27] M. Jin, Q. Huang, K. Zhao, P. Shang, *Int. J. Biol. Macromol.* **2013**, *54*, 16.
[28] R. J. Redgwell, D. Curti, J. K. Wang, J. M. Dobruchowska, G. J. Gerwig, J. P. Kamerling, P. Bucheli, *Carbohydr. Polym.* **2011**, *84*, 1344.
[29] M. A. Schell, M. Karmirantzou, B. Snel, D. Vilanova, B. Berger, G. Pessi, M. C. Zwahlen, F. Desiere, P. Bork, M. Delley, R. D. Pridmore, F. Arigoni, *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 14422.
[30] J. O. Lindsay, K. Whelan, A. J. Stagg, P. Gobin, H. O. Al-Hassi, N. Rayment, M. A. Kamm, S. C. Knight, A. Forbes, *Gut* **2006**, *55*, 348.
[31] C. Caballero-Franco, K. Keller, C. De Simone, K. Chadee, *Am. J. Physiol. Gastrointest. Liver Physiol.* **2007**, *292*, G315.
[32] B. Willing, J. Halfvarson, J. Dicksved, M. Rosenquist, G. Jarnerot, L. Engstrand, C. Tysk, J. K. Jansson, *Inflamm. Bowel Dis.* **2009**, *15*, 653.
[33] X. Sun, M. J. Zhu, *Mol. Nutr. Food Res. 62*, **2018**, 1700932.
[34] H. Liu, J. Wang, T. He, S. Becker, G. Zhang, D. Li, X. Ma, *Adv. Nutr.* **2018**, *9*, 21.
[35] A. Barcenilla, S. E. Pryde, J. C. Martin, S. H. Duncan, C. S. Stewart, C. Henderson, H. J. Flint, *Appl. Environ. Microbiol.* **2000**, *66*, 1654.
[36] M. Zhang, X. Qiu, H. Zhang, X. Yang, N. Hong, Y. Yang, H. Chen, C. Yu, *PLoS One* **2014**, *9*, e109146.
[37] M. Zhang, Q. Zhou, R. G. Dorfman, X. Huang, T. Fan, H. Zhang, J. Zhang, C. Yu, *BMC Gastroenterol.* **2016**, *16*, 84.
[38] P. Louis, H. J. Flint, *Appl. Environ. Microbiol.* **2007**, *73*, 2009.
[39] A. Belenguer, S. H. Duncan, A. G. Calder, G. Holtrop, P. Louis, G. E. Lobley, H. J. Flint, *Appl. Environ. Microbiol.* **2006**, *72*, 3593.
[40] R. Kuhn, J. Lohler, D. Rennick, K. Rajewsky, W. Muller, *Cell* **1993**, *75*, 263.
[41] E. Zigmond, S. Jung, *Trends Immunol.* **2013**, *34*, 162.
[42] S. Fujino, A. Andoh, S. Bamba, A. Ogawa, K. Hata, Y. Araki, T. Bamba, Y. Fujiyama, *Gut* **2003**, *52*, 65.
[43] L. A. Feagins, *Inflamm. Bowel Dis.* **2010**, *16*, 1963.
[44] E. Bettelli, Y. Carrier, W. Gao, T. Korn, T. B. Strom, M. Oukka, H. L. Weiner, V. K. Kuchroo, *Nature* **2006**, *441*, 235.
[45] I. I. Ivanov, L. Frutos Rde, N. Manel, K. Yoshinaga, D. B. Rifkin, R. B. Sartor, B. B. Finlay, D. R. Littman, *Cell Host Microbe* **2008**, *4*, 337.
[46] M. W. Babyatsky, G. Rossiter, D. K. Podolsky, *Gastroenterology* **1996**, *110*, 975.
[47] T. Fujii, Y. Ohtsuka, T. Lee, T. Kudo, H. Shoji, H. Sato, S. Nagata, T. Shimizu, Y. Yamashiro, *J Pediatr Gastroenterol Nutr.* **2006**, *43*, 83.
[48] F. Hoentjen, G. W. Welling, H. J. Harmsen, X. Zhang, J. Snart, G. W. Tannock, K. Lien, T. A. Churchill, M. Lupicki, L. A. Dieleman, *Inflamm. Bowel Dis.* **2005**, *11*, 977.
[49] B. Zheng, J. van Bergenhenegouwen, S. Overbeek, H. J. van de Kant, J. Garssen, G. Folkerts, P. Vos, M. E. Morgan, A. D. Kraneveld, *PLoS One* **2014**, *9*, e95441.
[50] A. Macpherson, U. Y. Khoo, I. Forgacs, J. Philpott-Howard, I. Bjarnason, *Gut* **1996**, *38*, 365.
[51] P. Brandtzaeg, H. S. Carlsen, T. S. Halstensen, *Adv. Exp. Med. Biol.* **2006**, *579*, 149.
[52] J. Vulevic, A. Juric, G. Tzortzis, G. R. Gibson, *J. Nutr.* **2013**, *143*, 324.
[53] J. P. Segain, D. Raingeard de la Bletiere, A. Bourreille, V. Leray, N. Gervois, C. Rosales, L. Ferrier, C. Bonnet, H. M. Blottiere, J. P. Galmiche, *Gut* **2000**, *47*, 397.
[54] F. Lasitschka, T. Giese, M. Paparella, S. R. Kurzhals, G. Wabnitz, K. Jacob, J. Gras, K. A. Bode, A. K. Heninger, T. Sziskzai, Y. Samstag, C. Leszinski, B. Jocher, M. Al-Saeedi, S. C. Meuer, J. Schroder-Braunstein, *Immun. Inflamm. Dis.* **2017**, *5*, 480.
[55] N. Singh, A. Gurav, S. Sivaprakasam, E. Brady, R. Padia, H. Shi, M. Thangaraju, P. D. Prasad, S. Manicassamy, D. H. Munn, J. R. Lee, S. Offermanns, V. Ganapathy, *Immunity* **2014**, *40*, 128.
[56] L. Peng, Z. R. Li, R. S. Green, I. R. Holzman, J. Lin, *J. Nutr.* **2009**, *139*, 1619.
[57] K. Lewis, F. Lutgendorff, V. Phan, J. D. Soderholm, P. M. Sherman, D. M. McKay, *Inflamm. Bowel Dis.* **2010**, *16*, 1138.