SUPPLEMENTARY MATERIAL

MATERIALS AND METHODS

Histology and immunohistochemistry

Following euthanasia, a subset of three mice colons, randomly selected from each group, was fixed in 10% buffered formalin for 24 h at room temperature and then dehydrated, paraffin embedded, 4 µm cut and mounted on glass slides. Slides were stained with H&E using standard protocols and observed on light microscopy (LEICA DM1000, Leica Microsystem, Milan, Italy). Microscopic damage in the mouse colon was evaluated and scored in blind by two independent pathologists according to Geboes score.

Gene expression analysis by quantitative Real-Time (qRT)- PCR

Total RNA from murine and human tissues was purified, quantified, characterized and retrotranscribed. Final preparation of RNA was considered DNA- and protein-free if the integrity number (Bionalyzer 2100, Agilent) was greater than 8 relative to a 0–10 scale. Quantitative real-time PCR was carried out in a iCycleriQ5 system (Biorad, MI, Italy) by use of SYBR Green detection. Selective primers were designed using Allele-Id software version 7.0 (Biosoft International, Palo Alto, CA, USA) and synthesized by MWG-Biotech (HPLC purification grade) (see Supplementary Table S2). Each sample was amplified simultaneously in a quadruplicate in one-assay run (maximum DCt of replicate samples <0.5), and a standard curve from consecutive fivefold dilutions (100–0.16 ng) of a cDNA pool representative of all samples was included for PCR efficiency determination. Data normalization was performed by using as a control the ct from S16 and/or HPRT, both constitutively expressed proteins; differences in mRNA content between groups were calculated as normalized values by use of the 2^-ΔΔct formula.

Fecal microbiota analysis by 16S rRNA gene sequencing using Illumina technology

Fecal microbiota was analyzed on a subset of three mice randomly selected from each group. Fecal samples were collected at time points T0 (before the induction of the colitis) and T3 (three days after the induction of colitis by DNBS) from animals treated or not with CBDV (three consecutive days

treatment after DNBS) and quickly stored at -80°C. Bacterial genomic DNA was extracted from frozen fecal samples using the QIAamp DNA Stool Mini Kit (Qiagen) according to manufacturer's instructions. The quantity and quality of DNA was determined by spectrophotometric measurements (NanoDrop; Thermo Fisher Scientific Inc., Waltham, MA, USA), and all DNA samples were stored at -20˚C until further processing for sequencing. The V3-V4 region of the 16S rRNA gene from each DNA sample was amplified and prepared for sequencing according to the protocol 16S Metagenomic Sequencing Library Preparation for Illumina Miseq System. Barcoded amplicons were mixed in equal amounts based on concentrations determined by Qubit Fluorometer (Invitrogen; Thermo Fisher Scientific, Inc.) and library sizes were assessed using a Bioanalyzer DNA 1000 chip (Agilent Technologies Gmbh, Waldbronn, Germany). Normalized libraries (4 nM) were pooled, denatured with NaOH (0.2 N) for 5 min at room temperature, diluted to 10 pM with HT1 buffer (Illumina MiSeq v3 Reagent kit; Illumina, Inc., San Diego, CA, USA) and combined with 25% (v/v) denatured 10 pM PhiX (Illumina, Inc.), according to Illumina guidelines. Sequencing run was performed on the Illumina MiSeq system using v3 reagents for 2 x 281 cycles (Illumina, Inc.).

Metagenomic data analysis

V3-V4 16S rDNA FASTQ paired-end reads were pre-processed with PEAR [58] in order to assemble reads with an overlap of at least 40 nucleotides, and to retain high quality sequences (PHRED score ≥ 33) that were comprised between 400 and 500 bp. These reads were then processed with PRINSEQ [59] in order to obtain FASTA and QUAL files for further analyses. Pick of operational taxonomic units (OTUs), taxonomic assignment and diversity analyses were conducted using Quantitative Insights Into Microbial Ecology (QIIME, version 1.8.0) [60]. A closed reference-based OTU picking method was employed to obtain OTUs at 97% sequence similarity from Greengenes 16S gene database (GG; May 2013 version) [61]. The GG database was used to taxonomically classify the identified OTUs and to compute their distribution across different taxonomic levels. To avoid sample size biases in subsequent analyses, samples were normalized to 18,871 sequences/sample using a sequence rarefaction procedure. Species heterogeneity in each sample was assessed by employing

two Alpha diversity metrics (the number Observed species and the Shannon entropy) and compared using a two-sample permutation t-test, using 999 Monte Carlo permutations to compute p-values. Unweighted and weighted Unifrac distances were calculated to analyze OTUs diversity among sample communities (beta diversity). Species and Clostridium cluster classification was performed using SPecies level IdentificatioN of metaGenOmic amplicons package (SPINGO version 1.3) with default parameters on a representative sequence of each OUT [62].

Supplementary Table S1. Pediatric patients with a well-established diagnosis of ulcerative colitis (UC) were included and clinically scored using the Geboes Score

Clinical Characteristics of Children with UC	
Patient	**The Geboes Score (0-5)**
#1	4-5
#2	4
#3	3
#4	2
#5	3
#6	5

Supplementary Table S2. List of primers used in RT-PCR analysis

Gene name	**Forward (5'-3')**	**Reverse (5'-3')**

Murine IL1-β	TATACCTGTCCTGTGTAAA	TTGACTTCTATCTTGTTG
Murine IL10	TTATTACCTCTGATAATCT	CCATCATATAATATAATCTCC
Murine IL6	CCTGGAGTACATGAAGAA	TGGTTGAAGATATGAATTAGAGT
Murine MCP1-α	TTGTATTTGTGACTATTTATTCT	GGCATATTTATTACTTCTCTG
Murine TRPA1	GGAGATATGTGTAGATTAGAAG AC	TCGGAGGTTTGGATTTGC
Murine RPV1	CTACCTCGTGTTCTTGTT	AGGCAGTGAGTTATTCTTC
Murine TRPV2	AACAAAGGAAAGAATGAG	GGTAGTTGAGATTCACTTT
Murine TRPV3	AACACCAACATTGATAAC	AGAAGGACAAGAAGAAC
Murine TRPV4	AAAGACTTGTTCACGAAG	CACAGAGTAGATGAAGTAGAG
Murine β-actin	CCAGGCATTGCTGACAGG	TGGAAGGTGGACAGTGAGG
Murine HPRT	TTGACACTGGTAAAACAATGC	GCCTG TATCCAACACTTCG
Human TRPA1	ACCCAAATCTCCGAAACTTCAAC	CTCAAGCAAGACCTTCATCACC
Human S16	TCGGACGCAAGAAGACAGCGA	AGCGTGCGCGGCTCAATCAT

Supplementary Table S3. Alpha diversity for 16S rRNA gene sequences at 97% similarity from NGS analysis. Alpha diversity indexes are reported as mean ± SD

Group	Observed species	Shannon entropy	Good's coverage (%)
CTRL T0	713.00 ± 8.04	6.48 ± 0.24	98.70 ± 0.03
DNBS T0	653.97 ± 53.27	6.30 ± 0.24	98.78 ± 0.07
DNBS + CBDV T0	751.00 ± 25.96	6.85 ± 0.09	98.74 ± 0.04
CTRL T3	695.30 ± 64.21	6.36 ± 0.21	98.72 ± 0.17
DNBS T3	548.67 ± 106.93	5.74 ± 0.67	98.99 ± 0.19
DNBS + CBDV T3	695.30 ± 64.21	6.48 ± 0.24	98.93 ± 0.13

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