

Letter to the Editor

Exclusive enteral nutrition in active pediatric Crohn disease: Effects on intestinal microbiota and immune regulation

To the Editor:

Unbalanced interactions between the intestinal immune system and the environment underlie Crohn disease (CD), a chronic inflammation dominated by pathogenic T_H1 and T_H17 cells.¹ Exclusive enteral nutrition (EEN), the exclusive feeding of liquid formula over several weeks, is the first-line treatment for induction of remission in pediatric patients with CD and induces mucosal healing.² A striking characteristic of pediatric CD is the therapeutic response to EEN, which can be observed within days after start of the nutritional intervention.² However, at present, the mechanisms of action remain elusive. We hypothesized that EEN exerts immunoregulatory properties that may be linked to changes in fecal microbiota.

EEN as induction therapy is usually prescribed for 6 to 8 weeks.² To assess the early response to treatment, we studied 15 pediatric patients (8 boys; mean age 13.5 ± 2.2 years; 12 newly diagnosed; see Table E1 in this article's Online Repository at www.jacionline.org) before start (pre-EEN) and 3 weeks thereafter (EEN). In parallel to clinical assessment and immunology tests, we characterized fecal microbiota by using high-throughput 16S rRNA gene sequencing before, at 2 weeks, and before end of EEN (Fig 1, A).

In line with previous observations, 3 weeks of EEN rapidly reduced disease activity and inflammatory markers, such as erythrocyte sedimentation rate, C-reactive protein, fibrinogen, or neutrophils, and improved albumin and hemoglobin levels (Fig 1, B; see Table E2 in this article's Online Repository at www.jacionline.org). Reassessment by sigmoidoscopy after 3 weeks of EEN showed improvement, although not complete mucosal healing (Fig 1, C).

Because innate and T-cell-derived cytokines are crucial to CD inflammation,¹ we hypothesized that EEN is paralleled with a downregulation of proinflammatory cytokine production. Indeed, PBMCs isolated during EEN and stimulated with bacterial ligands LPS or flagellin secreted less prototypic inflammatory mediators IL-6, IL-8, IL-1 β , and T_H1-derived IFN- γ but the response pattern of TNF or IL-17 was not altered (Fig 1, D; see Fig E1, A, in this article's Online Repository at www.jacionline.org). Importantly, and in addition to reduction of proinflammatory responses, EEN enhanced the capacity of antiinflammatory IL-10 to suppress LPS-induced IL-6 in PBMCs (Fig 1, E).

Because suppression of inflammatory mediators did not result from numeric changes in peripheral blood monocytes or total lymphocytes (Table E2), we assessed changes in T_H-cell subsets directly after isolation from peripheral blood during EEN. Frequencies of CCR6+ (T_H17) or CRTH2+ (T_H2) cells remained unchanged, whereas EEN significantly increased relative and absolute numbers of FOXP3+ regulatory T (Treg) cells (Fig 1, F; see Fig E2, A and B, in this article's Online Repository at www.jacionline.org). Analysis of gut-homing T_H cells expressing α 4 β 7-integrins revealed similar changes (Fig E2, C-E). The ratio between peripheral and mucosal Treg cells is a sensitive marker for intestinal inflammation because Treg cells respond

to inflammatory stimuli such as TNF³ and recruitment into the intestine is driven by initiation and resolution of inflammation.^{4,5} Indeed, EEN reduced Treg cells in the lamina propria, reflecting the resolution of intestinal inflammation as indicated by the diminished local expression of proinflammatory cytokines (Fig 1, G, and Fig E1, B). A similar change in the migration pattern of Treg cells has been demonstrated for anti-TNF medication, which is known to induce marked healing of mucosal ulcerations.⁶

Recent studies have analyzed the intestinal microbiota under EEN by using PCR-based approaches or 16S rRNA gene sequencing and reported substantial shifts and marked high interindividual variability associated with nutritional intervention.⁷⁻⁹ Very recently, 2 additional studies using in-depth shotgun metagenome sequencing further support these findings.^{10,11} Consistently, in 8 patients providing complete sets of stool samples, we observed significantly altered fecal bacterial communities already after 2 weeks of EEN (Fig 2, A). Individual phylogenetic profiles before intervention showed that newly diagnosed patients separated from those with long-standing disease (patients E and N, Fig 2, A). At 2 weeks of EEN, bacterial profiles clustered significantly from pre-EEN. However, patient C displayed major shifts in bacterial profiles only at the end of the intervention and the profile of patient E grouped already at baseline with EEN samples. Disease duration, previous therapeutic interventions, grade or location of inflammation, as well as the overall instability of the microbiota in the inflamed gut¹² might contribute to these differences. EEN decreased the relative sequence abundance of Gram-negative bacteria belonging to the phylum *Bacteroidetes*, including members of the family *Bacteroidaceae*, *Porphyromonadaceae*, and *Rikenellaceae* (Fig 2, B), which agrees with results of previous studies.^{8,9} Whereas CD-associated microbiota is typically characterized by reduced complexity of Gram-positive bacteria within the phylum *Firmicutes*,¹³ EEN increased their relative sequence abundance, especially members of the family *Christensenellaceae* (Fig 2, B).

At the phylotype level, the data set contained a total of 271 operational taxonomic units (OTUs), ranging between 91 and 164 per sample. Shannon effective species counts ranged from 8 to 50 per sample and were not affected by dietary intervention (Fig 2, B). This contrasts with Kaakoush et al⁷ who recently reported that EEN-induced disease remission was accompanied by reduced numbers of OTUs. However, we used stringent parameters for filtering OTUs to minimize effects of spurious molecular species.¹⁴ Shannon effective diversity takes into account the evenness of OTU distribution, thereby focusing attention on ecologically most relevant species. Furthermore, we found no significant differences in the relative abundance of any prevalent and dominant single molecular species (OTUs) associated with EEN.

To provide an integrated view, we investigated individual-specific associations between fecal bacterial composition and clinical or immunological parameters. In an exploratory network of correlations, 3 weeks of sole dietary intervention linked clinical and immunological responses to the relative abundance of 14 taxonomic groups (Fig 2, C; see Table E3 in this article's Online Repository at www.jacionline.org). Interestingly, specific

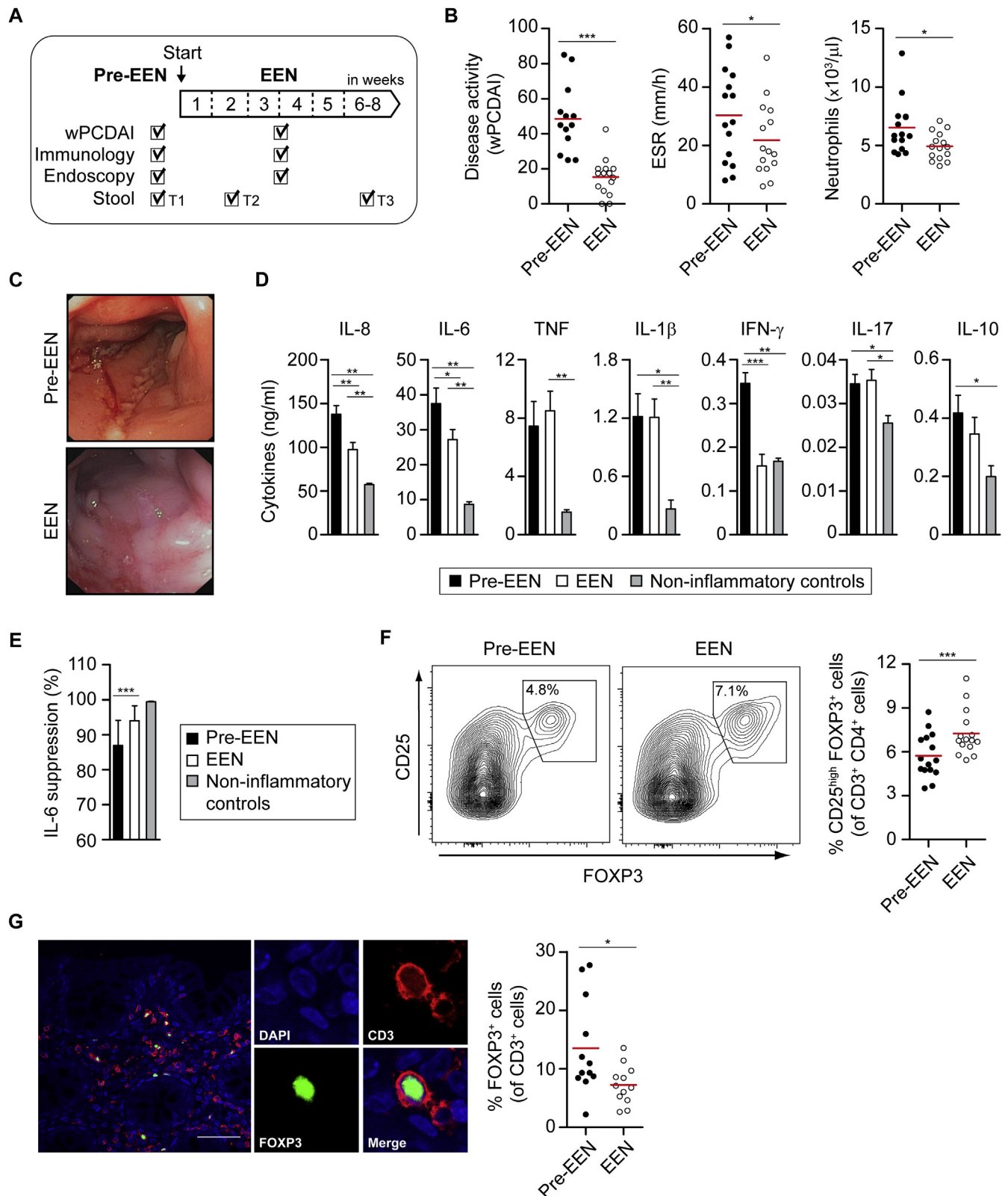
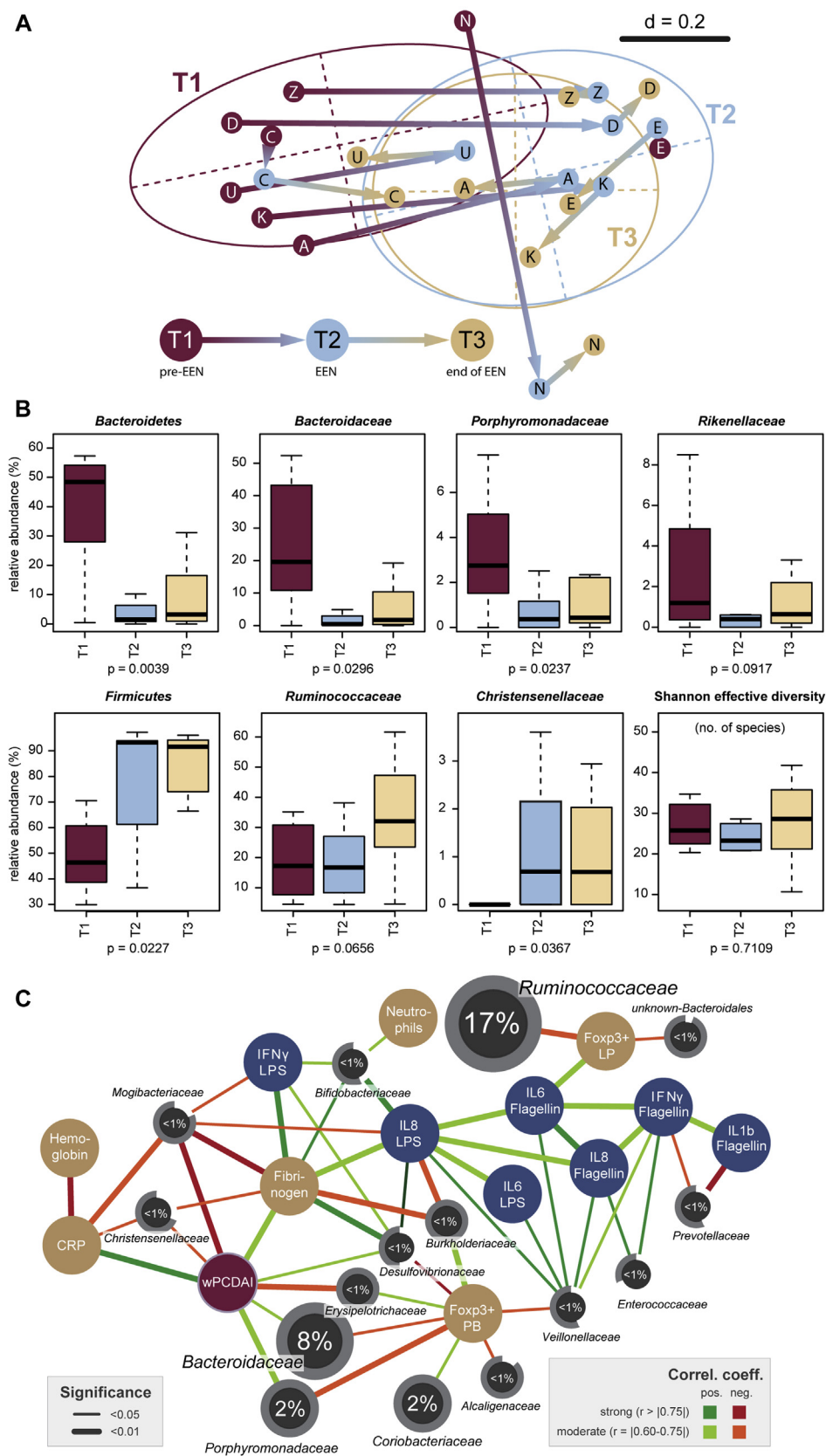


FIG 1. A, Prospective study design. Disease activity was measured by mathematically weighted Pediatric Crohn's Disease Activity Index (wPCDAI), which includes assessment of abdominal pain, general well-being, stools per day, erythrocyte sedimentation rate (ESR), albumin, weight loss, perirectal disease, and extraintestinal manifestations. Clinical parameters (B) and representative endoscopic appearance (C) before dietary intervention (Pre-EEN) and after 3 weeks of EEN. D, Cytokine production of LPS-stimulated PBMCs in patients (n = 14) and noninflammatory controls (n = 5). E, Suppression of IL-6 secretion by exogenous IL-10 in LPS-stimulated PMBCs. Data are normalized to LPS-induced IL-6 release in the absence of IL-10. F, Representative fluorescence-activated cell sorting blots and quantification of Treg-cell frequency. G, Lamina propria Treg cells in paired sections (n = 12, scale bar = 50 μm). DAPI, 4'-6-diamidino-2-phenylindole, dihydrochloride. * $P < .05$, ** $P < .01$, and *** $P < .001$.



microbial species with regulatory capacity on intestinal inflammation, such as induction of colonic Treg cells, are increasingly identified across different phyla.¹⁵ Because disease activity and Treg-cell pools correlated with several bacterial families, we speculate that EEN modulates inflammatory processes also through mechanisms mediated by the microbiota.

In conclusion, our data show that EEN induces rapid and complex changes in the fecal microbiota associated with normalized immune regulation and clinical improvement in pediatric CD. Although further studies are needed to elucidate mechanisms underlying EEN in more detail and in particular to determine causative relationships between the various factors observed, the present study provides first evidence of potential interactions between immune responses and the intestinal microbiota during EEN.

We thank the patients who participated in the study and their families for their support. We also thank the clinicians of the Department of Pediatric Gastroenterology (Folke Freudenberg, Mohammad-Samer Hajji, Kathrin Krohn, Christine Prell, Stephanie Barbara Schatz, and Andrea Schwarzer, in alphabetical order). We are also grateful to Caroline Ziegler and Sabrina Cabric for outstanding technical assistance with high-throughput sequencing.

Tobias Schwerd, MD^a

Klara Frivolt, MD^{a,b}

Thomas Clavel, PhD^c

Ilias Lagkouravdos, PhD^c

Gabor Katona, MD^a

Doris Mayr, MD^d

Holm H. Uhlig, MD, PhD^e

Dirk Haller, PhD^{c,f}

Sibylle Koletzko, MD^a

Philip Bufler, MD^a

From ^aDr von Hauner Children's Hospital, Ludwig-Maximilians-University, Munich, Germany; ^bthe 2nd Department of Pediatrics, Comenius University Medical School, Bratislava, Slovakia; ^cZIEL Institute for Food and Health, Technische Universität München, Freising-Weihenstephan, Germany; ^dthe Department of Pathology, Ludwig-Maximilians-University, Munich, Germany; ^ethe Translational Gastroenterology Unit and the Department of Paediatrics, University of Oxford, Oxford, United Kingdom; and ^fthe Chair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan, Germany. E-mail: tobias.schwerd@ndm.ox.ac.uk.

This work was supported by the LMU Munich (FöFoLe 742 to T.S.). K.F. was supported by the National Scholarship Program of the Slovak Republic, Collegium Talentum, and the SPP Foundation. H.H.U. is supported by the Crohn's and Colitis Foundation of America and the Leona M. and Harry B. Helmsley Charitable Trust. P.B. is supported by the German Research Foundation (DFG BU 1222/3-3).

Disclosure of potential conflict of interest: T. Schwerd has received a Young Investigator grant of the Ludwig-Maximilians-University Munich (FöFoLe), has received payment for lectures from Merck Sharp & Dohme, has received speaker's fees from MSD, and has received travel support from Nestlé Nutrition. K. Frivolt has received grants from the National Scholarship Program of the Slovak Republic, Collegium

Talentum, and the SPP Foundation and has received payment for lectures from Nestlé Nutrition. H. H. Uhlig has received grants from the Crohn's & Colitis Foundation of America and the Leona M. and Harry B. Helmsley Charitable Trust; has industrial project collaboration with Lilly, UCB Pharma, and Vertex Pharmaceuticals; has received payment for lectures from Actelion and Mitsubishi Tanabe Pharma Corporation; has received travel support from Actelion and MSD; and has a patent for coeliac disease diagnostics through Euroimmune. D. Haller has a board membership with Novonordisk and has received grants from the German Research Foundation and Helmsley. S. Koletzko has board memberships with MSD, Nestlé, Danone, and Merck; has consultant arrangements with Boehringer Ingelheim, MSA, Merck, AbbVie, and Danone; has received grants from Nestlé Nutrition, Mead Johnson, ThermoFisher, Eurospital, Euroimmun, INOVA, R-Biopharm, and Schär; has received payment for lectures from Centocor, MSD, Danone, Merck, Vifor, Nestlé Nutrition Institute, Euroimmun, ThermoFisher (Phadia), AbbVie, Schär, Hippi, and Falk; has received consulting or speaker's fees from AbbVie, Danone, Janssen Pharmaceutical Research & Development, Merck, MSD, Nestlé Nutrition, Vifor, and Weyth; has industrial project collaboration with Euroimmun, Eurospital, INOVA, Mead Johnson, Phadia-ThermoFisher, and Nestlé Nutrition; and has received travel support from MSD, Mead Johnson, and Nestlé Nutrition. P. Bufler has received grants from the German Research Foundation; has received lecture fees from MSD, Given Imaging, and AbbVie; had project collaboration with Euroimmun, INOVA, and ThermoFisher; and has received payment for lectures from MSD and AbbVie. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

- Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. *Nature* 2011;474:298-306.
- Ruemmele FM, Veres G, Kolho KL, Griffiths A, Levine A, Escher JC, et al. Consensus guidelines of ECCO/ESPGHAN on the medical management of pediatric Crohn's disease. *J Crohns Colitis* 2014;8:1179-207.
- Chen X, Wu X, Zhou Q, Howard OM, Netea MG, Oppenheim JJ. TNFR2 is critical for the stabilization of the CD4+Foxp3+ regulatory T cell phenotype in the inflammatory environment. *J Immunol* 2013;190:1076-84.
- Uhlig HH, Coombes J, Mottet C, Izcue A, Thompson C, Fanger A, et al. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. *J Immunol* 2006;177:5852-60.
- Mottet C, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol* 2003;170:3939-43.
- Boschetti G, Nancey S, Sardi F, Roblin X, Flourie B, Kaiserlian D. Therapy with anti-TNFalpha antibody enhances number and function of Foxp3(+) regulatory T cells in inflammatory bowel diseases. *Inflamm Bowel Dis* 2011;17:160-70.
- Kaakoush NO, Day AS, Leach ST, Lemberg DA, Nielsen S, Mitchell HM. Effect of exclusive enteral nutrition on the microbiota of children with newly diagnosed Crohn's disease. *Clin Transl Gastroenterol* 2015;6:e71.
- Gerasimidis K, Bertz M, Hanske L, Junick J, Biskou O, Aguilera M, et al. Decline in presumptively protective gut bacterial species and metabolites are paradoxically associated with disease improvement in pediatric Crohn's disease during enteral nutrition. *Inflamm Bowel Dis* 2014;20:861-71.
- Leach ST, Mitchell HM, Eng WR, Zhang L, Day AS. Sustained modulation of intestinal bacteria by exclusive enteral nutrition used to treat children with Crohn's disease. *Aliment Pharmacol Ther* 2008;28:724-33.
- Quince C, Ijaz UZ, Loman N, Eren AM, Saulnier D, Russell J, et al. Extensive modulation of the fecal metagenome in children with Crohn's disease during exclusive enteral nutrition. *Am J Gastroenterol* 2015;110:1718-29.

FIG 2. A, Interprofile relationships based on generalized UniFrac distances are depicted as a nonparametric multidimensional scaling (NMDS) plot. Stool samples were collected before initiation of EEN (T1), at 2 weeks (T2), and before the end of EEN (T3) and analyzed by using high-throughput 16S rRNA gene sequencing. Individual samples are shown with corresponding patient coding letter. **B**, Relative sequence abundances of specific taxa and Shannon effective diversity (lower panel, right). **C**, Network showing significant ($P < .05$) and substantial correlations (strong $r > |0.75|$; moderate $r = |0.60-0.75|$) between clinical parameters and bacterial taxa. Size of taxa nodes corresponds to the average relative abundance of the given taxon (as percentage of total sequences) across all samples in which it was detected. The outer ring indicates the number of samples that were positive for the particular taxon (samples from T1 and T2). Nodes were placed to allow good visualization of highly connected nodes, independent of whether correlations were negative or positive. The length of edges has no meaning and does not reflect any parameters of the correlations. Purple: disease activity by WPCDAI; blue: PBMC stimulation with LPS or flagellin; brown: ex vivo biochemistry/fluorescence-activated cell sorting; gray: microbiota. WPCDAI, Weighted Pediatric Crohn's Disease Activity Index.

11. Lewis JD, Chen EZ, Baldassano RN, Otley AR, Griffiths AM, Lee D, et al. Inflammation, antibiotics, and diet as environmental stressors of the gut microbiome in pediatric Crohn's disease. *Cell Host Microbe* 2015;18:489-500.
12. Dalal SR, Chang EB. The microbial basis of inflammatory bowel diseases. *J Clin Invest* 2014;124:4190-6.
13. Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a meta-genomic approach. *Gut* 2006;55:205-11.
14. Lagkouravdos I, Klaring K, Heinzmann SS, Platz S, Scholz B, Engel KH, et al. Gut metabolites and bacterial community networks during a pilot intervention study with flaxseeds in healthy adult men. *Mol Nutr Food Res* 2015;59:1614-28.
15. Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. *Cell* 2014;157:121-41.

<http://dx.doi.org/10.1016/j.jaci.2015.12.1331>

ONLINE REPOSITORY**TITLE PAGE**

Letter to the editor

Exclusive enteral nutrition in acute pediatric Crohn's disease: effects on intestinal microbiota and immune regulation

Tobias Schwerd, MD,^a Klara Frivolt, MD,^{a,b} Thomas Clavel, PhD,^c Ilias Lagkourdos, PhD,^c
Gabor Katona,^a Doris Mayr, MD,^d Holm H. Uhlig, MD, PhD,^e Dirk Haller, PhD,^{c,f} Sibylle
Koletzko, MD,^a and Philip Bufler, MD^a

^aDr. von Hauner Children's Hospital, Ludwig-Maximilians-University, Munich, Germany.

^b2nd Department of Pediatrics, Comenius University Medical School, Bratislava, Slovakia.

^c*ZIEL* Institute for Food and Health, Technische Universität München, Freising-Weihenstephan, Germany.

^dDepartment of Pathology, Ludwig-Maximilians-University, Munich, Germany.

^eTranslational Gastroenterology Unit, and Department of Paediatrics, University of Oxford, UK.

^fChair of Nutrition and Immunology, Technische Universität München, Freising-Weihenstephan, Germany.

Corresponding author:

Tobias Schwerd, M.D.

Present address: Translational Gastroenterology Unit, Experimental Medicine Division,

- 27 John Radcliffe Hospital, University of Oxford, Oxford OX3 9DU, England, UK
- 28 Telephone: +44 (0) 1865 220663
- 29 Fax: +44 (0)1865 222901
- 30 E-mail: tobias.schwerd@ndm.ox.ac.uk

METHODS

Study design

A total of 15 consecutive pediatric Crohn's disease (CD) patients with active inflammation were recruited over an 18 months period at our department. Patients were newly diagnosed and either therapy-naïve or on stable medical treatment, defined as azathioprine or 5'-ASA for at least three months. The diagnosis of CD was made according to revised Porto criteria.^{E1} Patient assessment was performed at time of diagnosis/relapse (defined as Pre-EEN) and 3-4 weeks after start of nutritional treatment (defined as EEN). Evaluation included medical history and physical examination, standard laboratory measures, calculation of the mathematically weighted Pediatric Crohn's Disease Activity Index (wPCDAI, includes assessment of abdominal pain, general well-being, stools per day, ESR, albumin, weight, perirectal disease, extra-intestinal manifestations),^{E2} anthropometric data, endoscopy and collection of heparinized blood. At time of diagnosis the IBD work-up included an ileocolonoscopy and was followed by a sigmoidoscopy for re-evaluation at three weeks of EEN. Stool samples were collected before initiation of EEN (T1), at two weeks (T2) and before end of EEN treatment (T3). Samples were stored immediately at -20°C in a home freezer and transferred on to the lab without defrosting. Eight patients provided appropriately stored stool samples across all time points. None of these patients received antibiotic treatment within three months prior to EEN initiation.

For PBMC stimulation studies, five children with non-inflammatory conditions served as controls (4 male, mean age 11 ± 3.8 years). The study protocol was approved by the local ethics committee (approval no. 411/11) and registered at the German Clinical Trials Register (accession no. DRKS00003548; registration 17/02/2012). Written informed consent was obtained from the parents and children older than 12 years signed a statement of assent.

Enteral nutritional therapy was prescribed as exclusive source of nutrition with Modulen IBD (Nestle, Vevey, Switzerland) or Neocate (Nutricia, Erlangen, Germany) as described previously.^{E3} Additionally only water was allowed. After diagnosis EEN was initiated in an inpatient setting with stepwise increase of formula over 2-3 days to exclude intolerance of formula and patient non-compliance. In therapy-naïve patients, immunomodulatory therapy (e.g. azathioprine) was not introduced before the re-evaluation visit.

Cell culture, stimulation and cytokine measurement

Human PBMC were freshly isolated within four hours from whole blood by Ficoll-Hypaque density gradient centrifugation. Cells were cultured at a density of 2×10^6 /ml in RPMI medium (Gibco) supplemented with 10% fetal calf serum. Cells were stimulated with LPS (10 ng/ml, Sigma, E coli O55:B5), flagellin (200 ng/ml, Invivogen) or a combination of LPS plus IL10 (5 ng/ml, Peprotech). Cytokines were measured in cell culture supernatant after 18 hours using the human Bio-Plex Pro Cytokine Kit, according to the manufacturer's instructions (Bio-Rad, Munich, Germany). Results for IL-6 were confirmed with a human IL-6 ELISA (BD biosciences). IL-8 and IL-1 β were measured by ELISA (BD biosciences).

Flow cytometry

To prevent in vitro stimulation effects on surface markers and transcription factors all samples were processed in less than four hours. Isolated PBMC were surface stained for CD3 (clone UCHT1, eBioscience), CD4 (clone SK3, BD Biosciences), CD25 (clone 2A3, BD Biosciences), CCR6 (clone 11A9, BD Biosciences), CCR2 (clone BM16, BD Biosciences), integrin $\alpha 4$ /CD49d (clone 9F10, BD Biosciences), integrin $\beta 7$ (clone FIB504, BD Biosciences), followed by intracellular staining for Foxp3 (clone PCH101, eBioscience) using Foxp3 staining kit (eBiosciences). Cells were acquired with a FACS Canto II supported by DIVA software (both BD biosciences). Post-acquisition analysis was

performed with FlowJo (flowjo.com). Absolute cell numbers of target populations were calculated from total lymphocytes of routine complete blood count.

Tissue samples

Mucosal biopsies were sampled from the sigmoid colon and rectum at the region with macroscopic inflammation. Biopsies under nutritional therapy were taken from the same area that had been affected before initiation of therapy. For RNA extraction tissue specimens were directly immersed in RNA later (Quiagen), stored overnight at 4°C and at -20°C until further analysis.

Quantitative PCR analysis of tissue biopsies

Total RNA was extracted from colonic tissues, reverse transcribed and gene expression of IL-1 β , IL-4, IL-6, IL-8, IL-10, IL-12p35, IL-17A, IL-22, IL-23A, IL-37, TNF- α , IFN- γ as well as the housekeeping gene TBP quantified by real-time PCR using SYBR Green according to established standard protocols. Expression of cytokine mRNA was normalized to TBP expression. Relative gene expression was calculated using the $\Delta\Delta C_t$ -method.

Fluorescence immunohistochemistry

Four micrometer sections were prepared from formalin fixed, paraffin-embedded tissue specimens. Tissue sections were deparaffinised with HistoClear (National diagnostics), rehydrated and incubated at 85°C in antigen-retrieval solution pH 9 (Dako, Glostrup, Denmark) for 20 minutes. Endogenous peroxidase activity was blocked with 0.05% sodium azide and 3% H₂O₂, followed by blocking with donkey serum. For visualization of Treg cells, colonic biopsies were sequentially stained using Tyramide Signal Amplification (PerkinElmer). Slides were incubated with anti-CD3 (polyclonal rabbit, Dako) and signal detected by donkey anti-rabbit HRP and tyramide Cy3 amplification. HRP activity was

inhibited by H₂O₂ and sodium azide. Tissue sections were blocked with goat serum and subsequently incubated with rat anti-Foxp3 (clone PCH101, eBioscience), anti-rat HRP polymer (Nichirei) and tyramide FITC amplification. Nuclei were counterstained with 4', 6'-Diamidino-2-phenylindole (DAPI) in order to recognize tissue structure and to confirm nuclear localization of Foxp3 transcription factor.

Dependent on availability, between one and three slides containing multiple biopsies from sigmoid colon and rectum were evaluated per patient. Biopsies were imaged completely on a FluoView FV1000 confocal microscope (Olympus) using a 20x/0.75 UPlanSAPO objective lens plus additional magnification of 1.6x by a XC30 camera (Olympus). Following the acquisition using FV10-ASW 3.0 software, biopsy images were reconstructed with Adobe Photoshop. Labelled cells were counted manually with ImageJ^{E4} by two independent researchers in five randomly chosen fields per slide.

High-throughput 16S ribosomal RNA (rRNA) gene sequencing

Fecal suspensions were allowed to melt on ice, homogenized, and a volume of 600 µL was added to 400 µL Phenol:Chloroform:IsoAmyl alcohol (25:24:1; Sigma-Aldrich), and 500 mg autoclaved 0.1 mm glass beads (Roth). Microbial cells were disrupted by mechanical lysis (3 x 30 sec at maximum speed) using a FastPrep®-24 (MP Biomedicals) fitted with a 24 x 2 mL cooling adaptor. After heat treatment (95°C, 5 min) and centrifugation (15.000 x g, 5 min, 4°C), supernatants were treated with RNase (0.1 µg/µL) for 30 min at 37°C. Metagenomic DNA was purified using gDNA columns (Macherey-Nagel) following the manufacturer's instructions. Concentrations and purity were controlled using the NanoDrop® system (Thermo Scientific) and samples were stored at 4°C during library preparation and at -20°C thereafter for longer storage.

The V3/V4 region of 16S rRNA genes was amplified (25 cycles) from 24 ng of metagenomic DNA using the bacteria-specific primers 341F and 785R^{E5} following a 2-step procedure to limit amplification bias.^{E6} Amplicons were purified using the AMPure XP system (Beckmann), pooled in an equimolar amount and sequenced in paired-end modus (PE275) using a MiSeq system (Illumina Inc.) following the manufacturer's instructions and a final DNA concentration of 10 pM and 15 % (v/v) PhiX standard library.

Sequence analysis

Raw read files were demultiplexed (allowing a maximum of 2 errors in barcodes) and each sample was processed using usearch^{E7} following the UPARSE approach.^{E8} First, all reads were trimmed to the position of the first base with quality score <3 and then paired. The resulted sequences were size filtered excluding those with assembled size <380 and >440 nucleotides. Paired reads with expected error >3 were further filtered out and the remaining sequences were trimmed by 10 nucleotides on each side to avoid GC bias and non-random base composition. For each sample, sequences were de-replicated and checked for chimeras with UCHIME.^{E9} Sequences from all samples were merged, sorted by abundance, and operational taxonomic units (OTUs) were picked at a threshold of 97% similarity. Finally, all sequences were mapped back to the representative sequences resulting in one OTU table for all samples.

Only those OTUs with a relative abundance above 0.25% total sequences in at least one sample were kept. RDP classifier^{E10} was used to assign taxonomic classification to the OTUs representative sequences (80% confidence). A phylogenetic tree was constructed using the Maximum Likelihood method in MEGA6.^{E11} Sequence proportions per sample were normalized to the mean sequence depth and distances between microbial profiles were calculated using the generalized Unifrac procedure.^{E12} For estimation of diversity within

samples (alpha-diversity), the Shannon index was calculated and transformed to the corresponding effective number of species as described by Jost.^{E13} Effective species counts reflect better the true diversity within samples when compared with metrics like species richness as they are less affected by the number of rare species.

We sequenced a total of 24 fecal samples (8 individuals across 3 time points) and analyzed a set of 1,467,666 sequences after quality- and chimera-check. The number of sequences per sample was $61,153 \pm 10,755$.

Statistics

Statistical analyses were performed in the R programming environment or GraphPad Prism version 5.00 (GraphPad Software, San Diego, CA, USA). For all tests, p-values below 0.05 were considered to be indicative of significant effects (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Cytokine results are depicted as mean $\pm$ SEM and statistical significance was determined by Wilcoxon test for paired cytokine data (Pre-EEN vs. EEN) or by Mann-Whitney-U test comparing cytokines from EEN patients to non-inflammatory controls. Clinical parameters and lymphocyte phenotype data are shown as individual data points. Red bar represents mean. Unless stated otherwise statistical significance was determined by paired t-test.

The effect of intervention on OTU and taxonomic counts was tested using one-way repeated measures ANOVA followed by paired t-test for pairwise comparisons. Prior to testing, individual counts $< 0.25\%$ were zeroed, and only dominant taxonomic groups or molecular species (i.e. median sequence abundance $> 1\%$ for at least one time point) were considered. Pearson correlation coefficients across all prevalent taxonomic groups (detected in at least one third of the samples) against clinical and immunological parameters were calculated using the log ratio-transformed and centered taxonomic composition table and visualized using the

187 Hmisc and corrgram packages. Abundances of zero were treated as missing values and thus
188 did not contribute to the correlations. For the network only significant ($p < 0.05$) and
189 substantial correlations (strong $r > |0.75|$; moderate $r = |0.60-0.75|$) are shown. The full dataset
190 including adjusted p-values is provided in Table E3. Correlation network was visualized in
191 Cytoscape 3.^{E14} For beta-diversity analysis, generalized Unifrac distances were calculated
192 using the package GUniFrac.^{E12}

References

- E1. Levine A, Koletzko S, Turner D, Escher JC, Cucchiara S, de Ridder L, et al. ESPGHAN revised porto criteria for the diagnosis of inflammatory bowel disease in children and adolescents. *J Pediatr Gastroenterol Nutr* 2014; 58:795-806.
- E2. Turner D, Griffiths AM, Walters TD, Seah T, Markowitz J, Pfefferkorn M, et al. Mathematical weighting of the pediatric Crohn's disease activity index (PCDAI) and comparison with its other short versions. *Inflamm Bowel Dis* 2012; 18:55-62.
- E3. Frivolt K, Schwerd T, Werkstetter KJ, Schwarzer A, Schatz SB, Bufler P, et al. Repeated exclusive enteral nutrition in the treatment of paediatric Crohn's disease: predictors of efficacy and outcome. *Aliment Pharmacol Ther* 2014; 39:1398-407.
- E4. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, et al. Fiji: an open-source platform for biological-image analysis. *Nat Methods* 2012; 9:676-82.
- E5. Klindworth A, Pruesse E, Schweer T, Peplies J, Quast C, Horn M, et al. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucleic Acids Res* 2013; 41:e1.
- E6. Berry D, Ben Mahfoudh K, Wagner M, Loy A. Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. *Appl Environ Microbiol* 2011; 77:7846-9.
- E7. Edgar RC. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 2010; 26:2460-1.
- E8. Edgar RC. UPARSE: highly accurate OTU sequences from microbial amplicon reads. *Nat Methods* 2013; 10:996-8.
- E9. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* 2011; 27:2194-200.

- 217 E10. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid
218 assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ
219 Microbiol 2007; 73:5261-7.
- 220 E11. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular
221 Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 2013; 30:2725-9.
- 222 E12. Chen J, Bittinger K, Charlson ES, Hoffmann C, Lewis J, Wu GD, et al. Associating
223 microbiome composition with environmental covariates using generalized UniFrac
224 distances. Bioinformatics 2012; 28:2106-13.
- 225 E13. Jost L. Partitioning diversity into independent *alpha* and *beta* components. Ecology
226 2007; 88:2427-39.
- 227 E14. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a
228 software environment for integrated models of biomolecular interaction networks.
229 Genome Res 2003; 13:2498-504.
- 230 E15. Ohnmacht C, Park JH, Cording S, Wing JB, Atarashi K, Obata Y, et al. MUCOSAL
231 IMMUNOLOGY. The microbiota regulates type 2 immunity through RORgammat(+) T
232 cells. Science 2015; 349:989-93.
- 233 E16. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM, et al.
234 MUCOSAL IMMUNOLOGY. Individual intestinal symbionts induce a distinct
235 population of RORgamma(+) regulatory T cells. Science 2015; 349:993-7.

236

237 **Table E1.** Baseline patient characteristics prior to EEN introduction

Baseline patient characteristics		Pre-EEN
Gender (male/female)		8/7
Age at manifestation (years)		12.0 ± 2.8
Age at diagnosis (years)		12.4 ± 2.9
Age at study inclusion (years)		13.5 ± 2.2
Positive family history		3/15 (20%)
Extraintestinal involvement		0/15
Disease location	L1 Terminal ileum	3/15 (20%)
	L2 Colon	1/15 (6.7%)
	L3 Ileocolonic	10/15 (67%)
	+ L4 (upper GI tract)	8/15 (53%)
Disease behavior	B1 nonstricturing-nonpenetrating	14/15 (73%)
	B2 stricturing	1/15 (6.7%)
	B3 penetrating	0/15
	Perianal involvement	6/15 (40%)
Newly-diagnosed and therapy-naïve		12/15 (80%)
Therapy-experienced		3/15 (20%)
Azathioprine		2/15 (13%)
5-Aminosalicylates		1/15 (6.7%)
Mild disease (wPCDAI ≤40)		4/15 (27%)
Moderate disease (wPCDAI >40≤57.5)		8/15 (53%)
Severe disease (wPCDAI >57.5)		3/15 (20%)
Modulen IBD/Neocate		14/1

EEN therapy duration until 1st follow-up visit (days)	25 ± 4
Weight (z-score) Pre-EEN	-0.85 ± 1.3
Weight (z-score) EEN	-0.67 ± 1.2

238

239 Data are expressed as mean $\pm$ standard deviation. EEN, exclusive enteral nutrition; wPCDAI,
240 mathematically weighted Pediatric Crohn's Disease Activity Index; IBD, inflammatory bowel
241 disease.

242 **Table E2.** Additional laboratory indices

Additional Laboratory indices	Pre-EEN	EEN	p-value
CRP (mg/dl), mean $\pm$ s.d.	2.0 $\pm$ 2.5	0.6 $\pm$ 0.9	0.0017 #
median (range)	1.2 (0.1; 9.4)	0.2 (0.1; 2.7)	
Fibrinogen (mg/dl), mean $\pm$ s.d.	443 $\pm$ 113	293 $\pm$ 81	0.0001
median (range)	445 (293; 616)	277 (185; 481)	
Albumin (g/dl), mean $\pm$ s.d.	3.7 $\pm$ 0.6	4.2 $\pm$ 0.5	0.0008
median (range)	3.9 (2.9; 4.7)	4.3 (3.2; 4.8)	
Hemoglobin (g/dl), mean $\pm$ s.d.	11.8 $\pm$ 1.7	12.5 $\pm$ 1.0	0.042
median (range)	12.3 (7.5; 14.4)	12.7 (10.1; 13.8)	
White blood count (cells/ μ l), mean $\pm$ s.d.	9373 $\pm$ 2713	7840 $\pm$ 1582	0.0215 #
median (range)	8900 (6600; 17400)	7700 (5900; 10300)	
Eosinophils (cells/ μ l), mean $\pm$ s.d.	255 $\pm$ 172	361 $\pm$ 334	0.2293 #
median (range)	231 (0; 693)	236 (63; 1106)	
Monocytes (cells/ μ l), mean $\pm$ s.d.	876 $\pm$ 380	716 $\pm$ 225	0.0630
median (range)	781 (89; 1740)	707 (413; 1740)	
Lymphocytes (cells/ μ l), mean $\pm$ s.d.	1702 $\pm$ 405	1807 $\pm$ 382	0.3757
median (range)	1764 (924; 2262)	1725 (1309; 2646)	
Platelets (G/l), mean $\pm$ s.d.	427 $\pm$ 125	394 $\pm$ 109	0.112
median (range)	425 (219; 653)	428 (521; 195)	
Immunoglobulin E IU/ml, mean $\pm$ s.d.	326.5 $\pm$ 766.5	246.8 $\pm$ 539.1	0.0151 #
median (range)	44.1 (0.1; 3001)	31.6 (0.2; 2111)	
ASCA+	10/15 (67%)		

243

244 Statistical significance was determined with Wilcoxon signed rank test (#) for non-parametric

245 data or paired t test for parametric parameters. Significant values are written in bold.

246

247

248 **Table E3**

	Value 1	Value 2	Correlation	P-value	P-value corr
1	wPCDAI	PB Foxp3+ cells	-0,40317377	0,0333896	0,1824
2	wPCDAI	CRP	0,760548532	2,64E-06	1,00E-04
3	wPCDAI	Fibrinogen	0,73062247	5,03E-05	0,0019
4	wPCDAI	Hemoglobin	-0,59206545	0,000903255	0,022
5	wPCDAI	Neutrophils	0,575724661	0,001347374	0,0294
6	wPCDAI	IL-8 LPS	0,4680852	0,012004283	0,1013
7	wPCDAI	IFN- γ LPS	0,584943116	0,001078029	0,0248
8	wPCDAI	IFN- γ Flagellin	0,421070248	0,028723757	0,1711
9	wPCDAI	<i>Alcaligenaceae</i>	0,549936652	0,041609255	0,2025
10	wPCDAI	<i>Bacteroidaceae</i>	0,618859768	0,010590313	0,0957
11	wPCDAI	<i>Burkholderiaceae</i>	-0,56308222	0,023142442	0,146
12	wPCDAI	<i>Christensenellaceae</i>	-0,70193416	0,016048339	0,1179
13	wPCDAI	<i>Desulfovibrionaceae</i>	0,740591824	0,03559058	0,1883
14	wPCDAI	<i>Erysipelotrichaceae</i>	-0,64749217	0,006693178	0,0771
15	wPCDAI	<i>Mogibacteriaceae</i>	-0,80702603	0,000854105	0,0214
16	wPCDAI	<i>Porphyromonadaceae</i>	0,692614973	0,002940074	0,0487
17	PB Foxp3+ cells	IFN- γ LPS	-0,52370518	0,0042344	0,0601
18	PB Foxp3+ cells	IFN- γ Flagellin	-0,41448432	0,031589767	0,1819
19	PB Foxp3+ cells	<i>Alcaligenaceae</i>	-0,62787592	0,016207606	0,118
20	PB Foxp3+ cells	<i>Bacteroidaceae</i>	-0,62036216	0,010349391	0,0957
21	PB Foxp3+ cells	<i>Burkholderiaceae</i>	0,632385492	0,008573125	0,0897
22	PB Foxp3+ cells	<i>Coriobacteriaceae</i>	0,616347909	0,011002957	0,0977
23	PB Foxp3+ cells	<i>Desulfovibrionaceae</i>	-0,81879258	0,012926996	0,1031
24	PB Foxp3+ cells	<i>Erysipelotrichaceae</i>	0,621292114	0,010202444	0,0957
25	PB Foxp3+ cells	<i>Lachnospiraceae</i>	0,517975032	0,039853983	0,198

26	PB Foxp3+ cells	<i>Porphyromonadaceae</i>	-0,64771932	0,006667658	0,0771
27	PB Foxp3+ cells	<i>Veillonellaceae</i>	-0,71017581	0,032052219	0,1821
28	IL-6 Flagellin	LP Foxp3+ cells	0,604028642	0,002911035	0,0487
29	IL-6 Flagellin	Fibrinogen	0,506806016	0,011490167	0,1009
30	IL-6 Flagellin	IL-6 LPS	0,529417515	0,003766723	0,0563
31	IL-6 Flagellin	IL-8 LPS	0,628658772	0,000339955	0,0097
32	IL-6 Flagellin	IFN- γ LPS	0,397572815	0,036163327	0,1885
33	IL-6 Flagellin	IL-8 Flagellin	0,84095329	2,11E-08	0
34	IL-6 Flagellin	IFN- γ Flagellin	0,689211905	7,01E-05	0,0022
35	IL-6 Flagellin	IL-1 β Flagellin	0,54077208	0,002966831	0,0487
36	IL-6 Flagellin	<i>Burkholderiaceae</i>	-0,56254739	0,023301998	0,146
37	IL-6 Flagellin	<i>Mogibacteriaceae</i>	-0,59123307	0,03332893	0,1824
38	IL-6 Flagellin	<i>Veillonellaceae</i>	0,793654323	0,010668995	0,0957
39	LP Foxp3+ cells	IL-8 Flagellin	0,460434288	0,031055476	0,18
40	LP Foxp3+ cells	<i>Ruminococcaceae</i>	-0,71123236	0,009499675	0,0957
41	LP Foxp3+ cells	<i>unknown_Bacteroidales</i>	-0,62605822	0,03933878	0,198
42	CRP	Fibrinogen	0,499485075	0,012953084	0,1031
43	CRP	Hemoglobin	-0,80696487	2,13E-07	0
44	CRP	Neutrophils	0,554286718	0,002208672	0,0409
45	CRP	<i>Christensenellaceae</i>	-0,64915115	0,030676191	0,179
46	CRP	<i>Mogibacteriaceae</i>	-0,71466649	0,00604873	0,0736
47	Fibrinogen	Hemoglobin	-0,51156068	0,010615165	0,0957
48	Fibrinogen	Neutrophils	0,437999189	0,032298014	0,1822
49	Fibrinogen	IL-6 LPS	0,421558589	0,040198002	0,198
50	Fibrinogen	IL-8 LPS	0,647992551	0,000617265	0,0164
51	Fibrinogen	IFN- γ LPS	0,753445685	2,14E-05	0,001
52	Fibrinogen	IL-8 Flagellin	0,54687959	0,005685108	0,073

53	Fibrinogen	IFN- γ Flagellin	0,49055779	0,017472905	0,122
54	Fibrinogen	<i>Bacteroidaceae</i>	0,567633688	0,034233739	0,1846
55	Fibrinogen	<i>Bifidobacteriaceae</i>	0,862145782	0,012564738	0,1029
56	Fibrinogen	<i>Burkholderiaceae</i>	-0,70426422	0,004926949	0,0666
57	Fibrinogen	<i>Christensenellaceae</i>	-0,74455184	0,02138032	0,1415
58	Fibrinogen	<i>Desulfovibrionaceae</i>	0,894862652	0,006500343	0,0771
59	Fibrinogen	<i>Mogibacteriaceae</i>	-0,8245979	0,000523711	0,0144
60	Hemoglobin	Neutrophils	-0,44792098	0,016834609	0,1205
61	Neutrophils	IL-8 LPS	0,398283958	0,035801245	0,1883
62	Neutrophils	IFN- γ Flagellin	0,489251465	0,00959914	0,0957
63	Neutrophils	<i>Bifidobacteriaceae</i>	0,706662536	0,033305736	0,1824
64	Neutrophils	<i>Mogibacteriaceae</i>	-0,56250775	0,04537258	0,2112
65	IL-6 LPS	IL-8 LPS	0,685845912	5,62E-05	0,002
66	IL-6 LPS	IL-8 Flagellin	0,458658695	0,014094474	0,1082
67	IL-6 LPS	<i>Burkholderiaceae</i>	-0,58844364	0,016491977	0,1191
68	IL-6 LPS	<i>Mogibacteriaceae</i>	-0,57144636	0,0413345	0,2024
69	IL-6 LPS	<i>Veillonellaceae</i>	0,774435341	0,014279037	0,1086
70	IL-8 LPS	IFN- γ LPS	0,521062553	0,004466972	0,0624
71	IL-8 LPS	IL-8 Flagellin	0,684322417	5,92E-05	0,002
72	IL-8 LPS	IFN- γ Flagellin	0,450230211	0,018445286	0,1257
73	IL-8 LPS	<i>Alcaligenaceae</i>	0,554330945	0,039678256	0,198
74	IL-8 LPS	<i>Bifidobacteriaceae</i>	0,847144961	0,003945914	0,0576
75	IL-8 LPS	<i>Burkholderiaceae</i>	-0,74476629	0,00093335	0,0221
76	IL-8 LPS	<i>Coriobacteriaceae</i>	-0,57743442	0,019165965	0,1296
77	IL-8 LPS	<i>Desulfovibrionaceae</i>	0,775346756	0,023783824	0,1468
78	IL-8 LPS	<i>Mogibacteriaceae</i>	-0,65609431	0,014881295	0,1122
79	IL-8 LPS	<i>Veillonellaceae</i>	0,783302724	0,012525661	0,1029

80	IFN- γ LPS	IL-1 β LPS	0,438803703	0,019493431	0,1308
81	IFN- γ LPS	IL-8 Flagellin	0,426480502	0,023626217	0,1468
82	IFN- γ LPS	IFN- γ Flagellin	0,565965772	0,0020905	0,0396
83	IFN- γ LPS	IL-1 β Flagellin	0,453443527	0,015375058	0,1149
84	IFN- γ LPS	<i>Bifidobacteriaceae</i>	0,689060509	0,040066013	0,198
85	IFN- γ LPS	<i>Burkholderiaceae</i>	-0,51091033	0,04313001	0,2064
86	IFN- γ LPS	<i>Desulfovibrionaceae</i>	0,748259187	0,03273298	0,1823
87	IFN- γ LPS	<i>Mogibacteriaceae</i>	-0,68264091	0,01013561	0,0957
88	IL-1 β LPS	IFN- γ Flagellin	0,382267535	0,049091557	0,2201
89	IL-1 β LPS	IL-1 β Flagellin	0,477565944	0,010169307	0,0957
90	IL-1 β LPS	<i>Ruminococcaceae</i>	-0,51992416	0,038984015	0,198
91	IL-8 Flagellin	IFN- γ Flagellin	0,733193398	1,36E-05	6,00E-04
92	IL-8 Flagellin	IL-1 β Flagellin	0,565490127	0,001712998	0,0347
93	IL-8 Flagellin	<i>Enterococcaceae</i>	0,883085251	0,047137993	0,2159
94	IL-8 Flagellin	<i>Veillonellaceae</i>	0,787034988	0,011833102	0,1013
95	IFN- γ Flagellin	IL-1 β Flagellin	0,704598963	4,08E-05	0,0017
96	IFN- γ Flagellin	<i>Enterococcaceae</i>	0,928798735	0,022561704	0,1435
97	IFN- γ Flagellin	<i>Prevotellaceae</i>	-0,74441063	0,021418479	0,1415
98	IFN- γ Flagellin	<i>Veillonellaceae</i>	0,743448138	0,021679786	0,1421
99	IL-1 β Flagellin	<i>Prevotellaceae</i>	-0,81679767	0,007207714	0,0798
100	IL-1 β Flagellin	<i>Ruminococcaceae</i>	-0,54894549	0,027651854	0,1659

249

250 Results summary of all significant correlations (strong $r > |0.75|$; moderate $r = |0.60-0.75|$;
 251 weak $r = |0.40-0.60|$) and adjusted p-values (marked in light grey). Raw data of correlation
 252 network are deposited online (please provide link).

Figure legends

Figure E1. EEN regulates cytokine responses in PBMC and colonic epithelial biopsies.

A, Freshly isolated PBMC of 14 patients with active IBD were stimulated at baseline (Pre-EEN) and after three weeks of EEN with flagellin. Cytokine concentrations were determined in cell culture supernatant 18 hours after stimulation. Data are compared to five pediatric non-inflammatory controls. IL-6, IL-4, IL-10, IL-17, TNF and IFN- γ were measured by LUMINEX technology; IL-1 β and IL-8 by ELISA. Data are depicted as mean $\pm$ SEM and statistical significance is calculated for paired cytokine data (Pre-EEN vs EEN, Wilcoxon test) or data under EEN and non-inflammatory controls (Mann-Whitney-U test). **B,** Paired colonic biopsies from 8 patients undergoing EEN were analyzed by quantitative PCR for changes in inflammatory cytokines. The relative expression of target genes was calculated by the $2^{(-\Delta\Delta Ct)}$ -Method for individual patients and depicted as mean $\pm$ SEM.

Figure E2. EEN modifies certain T helper cell subsets. **A,** Absolute numbers of Treg cells increase with EEN therapy. **B,** Ratio of Treg cells to naïve T effector (Teff) cells and activated Teff cells. The frequency of naïve and activated Teff cells remained unchanged. **C-E,** Changes in T helper cells were analyzed in the compartment of gut homing CD4⁺ T cells ($\alpha 4 + \beta 7$ high). Gating strategy is shown in representative FACS plots. **D,** EEN does not alter relative frequencies of Th17 (CCR6⁺) and Th2 (CRTH2⁺) cells in gut homing population. **E,** Increase in Treg cells and T cells co-expressing Foxp3 and CCR6 is found during EEN. Future studies are necessary to elucidate the origin and function of Foxp3⁺CCR6⁺ T cells during EEN. Recent literature would suggest that this subpopulation are gut homing Tregs that co-express Foxp3 and ROR γ t.^{E15, 16}

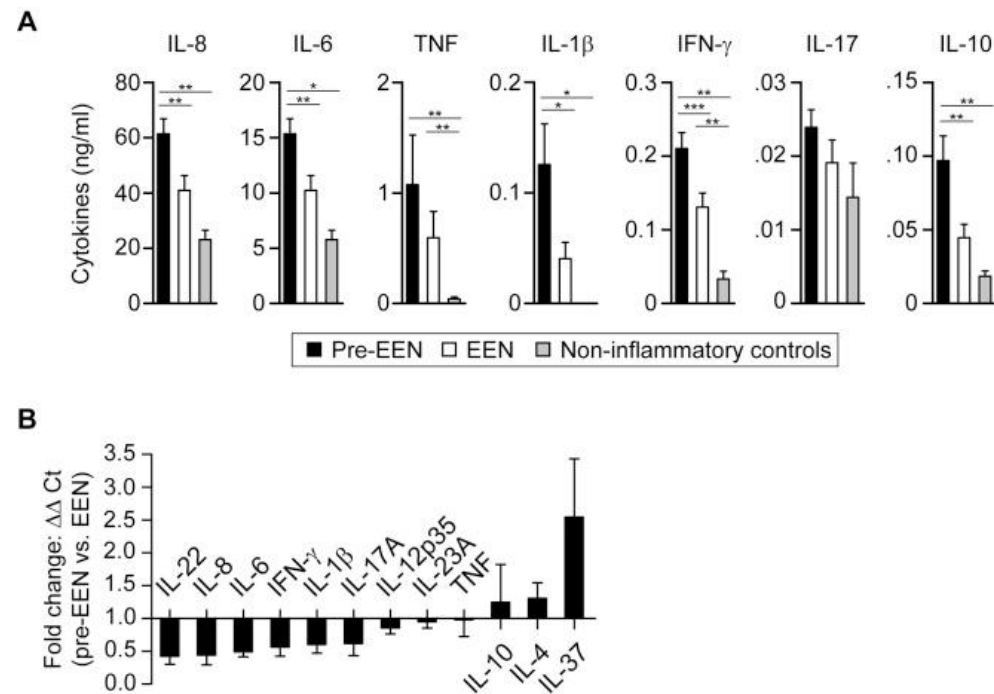


Fig E1.

Tobias Schwerd, Klara Frivolt, Thomas Clavel, Ilias Lagkourvardos, Gabor Katona, Doris Mayr, Holm H. Uhlig, Dirk Haller, Sibylle Koletzko, Philip Bufler

Exclusive enteral nutrition in active pediatric Crohn disease: Effects on intestinal microbiota and immune regulation

Journal of Allergy and Clinical Immunology, 2016, Available online 15 March 2016

<http://dx.doi.org/10.1016/j.jaci.2015.12.1331>

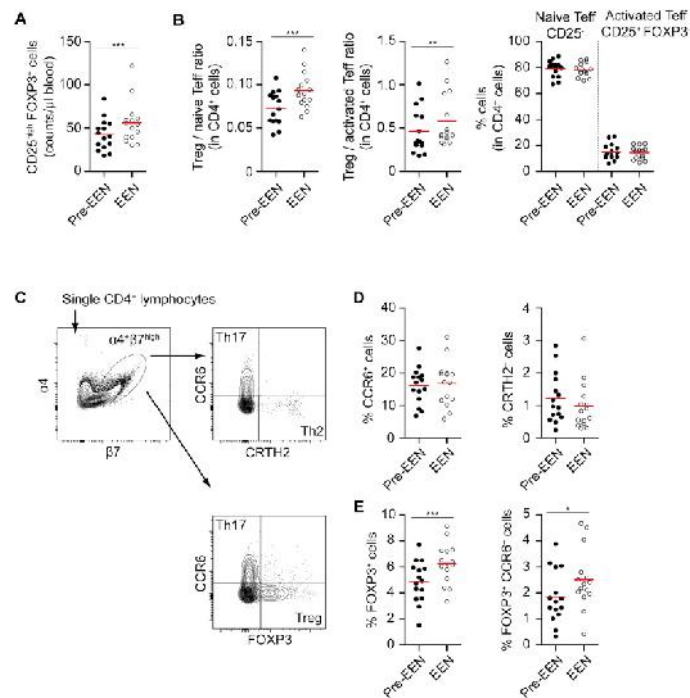


Fig E2.

Tobias Schwerd, Klara Frivolt, Thomas Clavel, Ilias Lagkouvardos, Gabor Katona, Doris Mayr, Holm H. Uhlig, Dirk Haller, Sibylle Koletzko, Philip Bufler

Exclusive enteral nutrition in active pediatric Crohn disease: Effects on intestinal microbiota and immune regulation

Journal of Allergy and Clinical Immunology, 2016, Available online 15 March 2016

<http://dx.doi.org/10.1016/j.jaci.2015.12.1331>