the NIH, serves as an advisory board member for Teva and a consultant for GlaxoSmithKline and received research funding from the NIH, AstraZeneca, GlaxoSmithKline, Sanofi, and Genentech. M. E. Wechsler personal fees from Novartis, Sepracor/Sunovion, NKT Therapeutics, Asthmatx/BSCI, Merck, Regeneron, MedImmune, Novartis, Ambitbio, Vectura, Sanofi, Teva, Boehringer Ingelheim, GlaxoSmithKline, and AstraZeneca, outside the submitted work. M. Castro reports research funding from the National Institutes of Health during the conduct of the study; personal fees from Asthmatx/Boston Scientific, IPS/Holaria, Genentech, Merck, GlaxoSmithKline, Genentech, Boehringer Ingelheim, and Elsevier; grants from Boston Scientific, Amgen, Ception/Cephalon/Teva, Genetech, MedImmune, Merck, Novartis, GlaxoSmithKline, Sanofi Aventis, Vectura, NextBio, and KalaBios; and stock options from Sparo, all outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

REFERENCES

1. Castro M, King TS, Kunselman SJ, Cabana MD, Denlinger L, Holguin F, et al. Effect of vitamin D_3 on asthma treatment failures in adults with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. JAMA 2014;311:2083-91.
2. Mulligan JK, Bleier BS, O'Connell B, Mulligan RM, Wagner C, Schlosser RJ. Vitamin D_3 correlates inversely with systemic dendritic cell numbers and bone erosion in chronic rhinosinusitis with nasal polyps and allergic fungal rhinosinusitis. Clin Exp Immunol 2011;164:312-20.
3. Mulligan JK, Nagel W, O'Connell BP, Wentzel J, Atkinson C, Schlosser RJ. Cigarette smoke exposure is associated with vitamin D_3 deficiencies in patients with chronic rhinosinusitis. J Allergy Clin Immunol 2014;134:342-9.
4. Dixon AE, Sugar EA, Zinreich SJ, Slavin RG, Corren J, Naclerio RM, et al. Criteria to screen for chronic sinonasal disease. Chest 2009;136:1324-32.
5. Pinto JM, Schneider J, Perez R, DeTineo M, Baroody FM, Naclerio RM. Serum 25-hydroxyvitamin D levels are lower in urban African American subjects with chronic rhinosinusitis. J Allergy Clin Immunol 2008;122:415-7.
6. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 2013;369:1991-2000.

Available online March 11, 2016.
http://dx.doi.org/10.1016/j.jaci.2015.12.1329

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-1β, 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 α4β7-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 TNF[3] 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 gut[12] 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

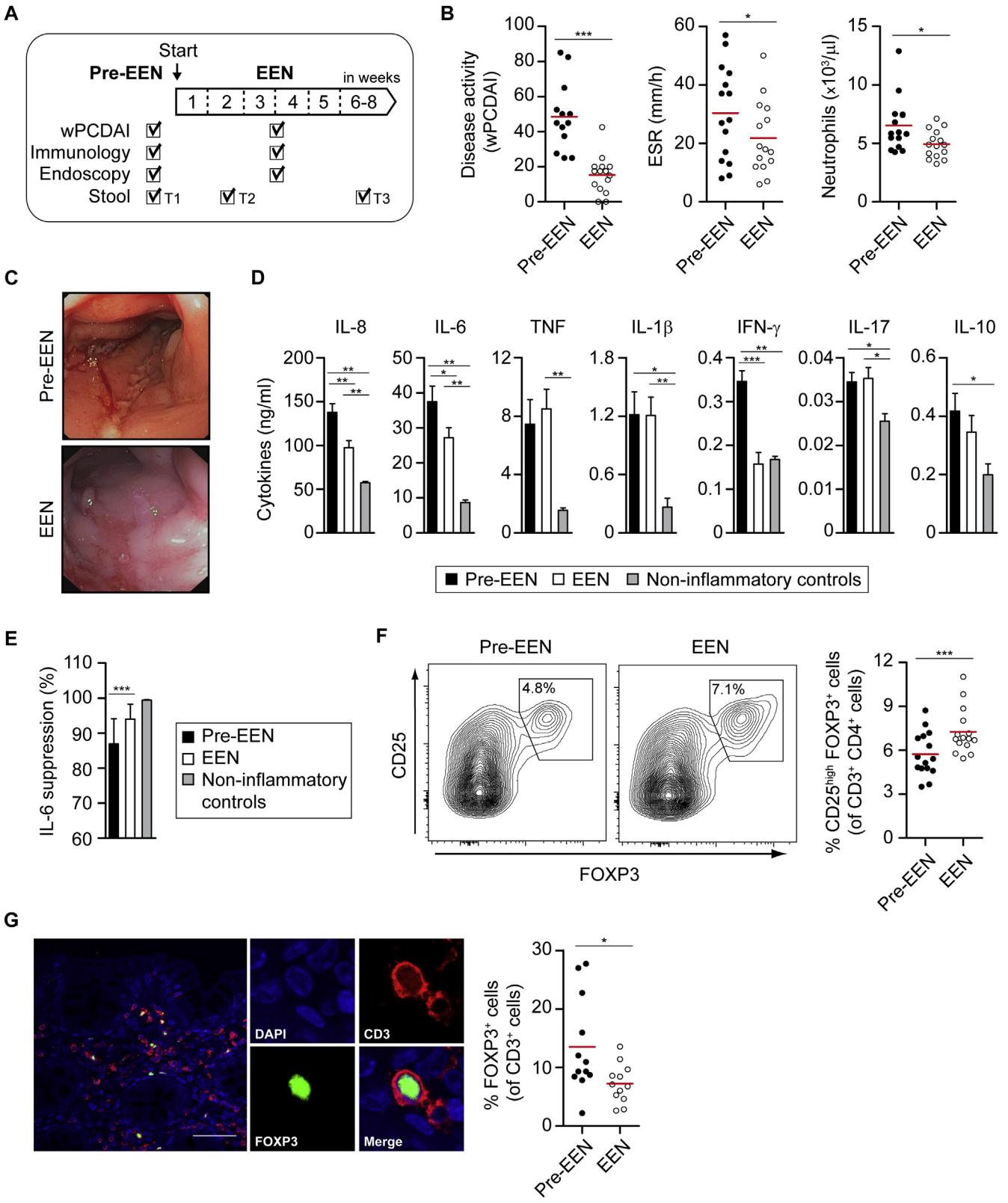

FIG 1. A, Prospective study design. Disease activity was measured by mathematically weighted Pediatric Crohn's Disease Activity Index (wPCDAI), which includes assessment of abdominal pain, general well-being, stools per day, erythrocyte sedimentation rate (ESR), albumin, weight loss, perirectal disease, 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.