

The Autoimmune Protocol Diet Modifies Intestinal RNA Expression in Inflammatory Bowel Disease

To the Editor,

Inflammatory bowel diseases (IBDs), including Crohn disease (CD) and ulcerative colitis (UC), are multifactorial intestinal inflammatory disorders attributed to gut dysbiosis, genetic susceptibility, and environmental triggers.¹ Environmental triggers, such as diet, tobacco use, infections, and antibiotics, are thought to activate the mucosal immune system, disrupt epithelial barrier function, and contribute to microbial dysbiosis, which, ultimately, may influence disease course.² With increasing interest in the role of diet in IBD, dietary modification has become a key target for the symptomatic and therapeutic modulation of IBD.

The autoimmune protocol (AIP) diet, an extension of the Paleolithic diet,³ involves elimination of foods that may act as antigens, stimulate mucosal inflammation, and/or trigger dysbiosis within the gastrointestinal tract.³ We recently published an uncontrolled single-center clinical trial to examine the efficacy of the AIP diet for adults with active CD and UC.⁴ The study was approved by the Scripps Institutional Review Board and registered on [ClinicalTrials.gov](https://clinicaltrials.gov) (identifier

NCT03512327). Enrollment was initiated on September 1, 2016, with study completion on December 10, 2016. Adults (>18 years of age) with CD and a Harvey-Bradshaw Index (HBI) score ≥ 5 , or UC and a partial Mayo score ≥ 3 , as well as objective evidence of active disease [ulcerations/erosions on endoscopy within 7 months and/or elevated fecal calprotectin (FC > 50 $\mu\text{g/g}$) within 1 month of enrollment], were eligible for the study. The AIP dietary protocol consisted of an initial 6-week elimination phase, with the staged elimination of grains, legumes, nightshades, dairy, eggs, coffee, alcohol, nuts and seeds, refined/processed sugars, oils, nonsteroidal anti-inflammatory drugs, and food additives. This was followed by a 5-week maintenance phase, during which food group reintroduction was not allowed. Counseling was provided by a health coach and registered dietitian, emphasizing inclusion of a nutrient-dense diet, fermented foods, meal ingredients, and preparation. A total of 18 adult participants were enrolled, but 3 withdrew before the start of the study, due to an inability to commit to the proposed diet. The final cohort included 9 participants with CD and 6 with UC; 47% of study participants were on biologic therapy, with or without concomitant immunomodulatory, at baseline; the rest were on mesalamine-based therapy (47%) or no medication. Overall, 73% of patients achieved clinical remission by week 6, and maintained remission through the 5-week maintenance phase.⁴ We also observed a nonsignificant trend toward reductions in FC.

To extend these data, we sought to determine whether dietary change influenced mucosal inflammation by examining changes in mucosal RNA expression at baseline and end of study. Among 15 participants, 7 patients underwent endoscopy pre- and post-diet; of these, only 5 participants (4 UC and 1 CD) had study biopsies collected for analysis at both time points. Due to the limited number with CD, we limited our examination of RNA expression to

UC. Here, we present the results of this substudy. To our knowledge, this is the first study correlating changes in mucosal RNA expression during dietary therapy in patients with active UC.

Four participants with UC underwent both pre- and post-AIP diet colonoscopy or sigmoidoscopy, and were included in the RNA substudy (Table 1). Among these patients, mean partial Mayo score improved from 5.5 (week 0) to 0.5 (week 6, $P < 0.01$) and 0.25 (week 11, $P < 0.01$). Mean Mayo endoscopic subscore (MES) improved from baseline 1.25 ± 0.50 to 0.50 ± 0.58 ($P = 0.058$) at the end of the study. Specifically, MES decreased by 1 point in 3 participants [MES 2 \rightarrow 1 ($n = 1$), MES 1 \rightarrow 0 ($n = 2$)] or did not change [MES 1 ($n = 1$)]. FC also decreased, from 414 (week 0) to 88 (week 6, $P = 0.2$) and 70 (week 11, $P = 0.2$). On histology, all four participants had chronic mild-moderately active colitis at baseline. Post-dietary intervention, 3 participants had chronic minimally mildly active colitis, and 1 participant had benign colonic mucosa.

Mucosal RNA expression was evaluated using RNA-seq on colonic tissue biopsy samples collected before and after dietary intervention. Total RNA was isolated from RNAlater stabilized tissue samples using Qiagen RNeasy Mini kit. Following total RNA isolation, RNA-Seq libraries were prepared using Illumina TruSeq RNA Sample Prep kit as per kit instructions. The libraries were enriched by performing 15 cycles PCR and finally pooled together and sequenced on HiSeq2500 Rapid Mode. The sequenced reads were first run through Trimmomatic⁵ to remove any adapter contamination. The trimmed reads were then aligned to the most recent human genome assembly hg38 using Bowtie2.⁶ Finally, read counts for genes were generated using HT-Seq,⁷ and only uniquely aligning reads were considered for this step.

To identify genes which were differentially expressed due to the AIP diet,

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TABLE 1. Participant Characteristics

	Total UC Cohort (n = 6)	RNA Sequencing UC (n = 4)
Age (years), mean (SD)	41 (15)	44 (19)
Female, n (%)	4 (67)	2 (50)
UC duration (years), mean (SD)	15.3 (14.6)	17.8 (17.7)
UC location	Rectum (n = 1) Left side (n = 2) Pancolitis (n = 3)	Rectum (n = 1) Left side (n = 1) Pancolitis (n = 2)
Tobacco use		
Never, n (%)	6 (100)	4 (100)
IBD medication use		
Mesalamine, n (%)	5 (83)	4 (100)
Immunomodulator, n (%)	0 (0)	0 (0)
Biologic, n (%)	1 (17)	0 (0)
Systemic steroid, n (%)	2 (33)	0 (0)
FC (μg/g), mean (range)	376 (25–1177)	414 (25–1177)

TABLE 2. Top 10 Biological Processes GO Terms Enriched in the Set of Downregulated and Upregulated Genes Based on the Combined Score Enrichment Metric

Downregulated GO Terms	Gene Count	P Value (Bonferroni)
GO:0006955~immune response	37	7.57E-006
GO:0009605~response to external stimulus	42	4.60E-005
GO:0006952~defense response	34	2.09E-004
GO:0006954~inflammatory response	21	4.85E-004
GO:1903524~positive regulation of blood circulation	9	1.50E-003
GO:0042127~regulation of cell proliferation	32	3.71E-003
GO:0050880~regulation of blood vessel size	10	4.34E-003
GO:0035150~regulation of tube size	10	4.61E-003
GO:0070887~cellular response to chemical stimulus	43	1.33E-002
GO:0003018~vascular process in circulatory system	10	1.76E-002
Upregulated GO Terms		
GO:0009891~positive regulation of biosynthetic process	37	4.48E-005
GO:0033993~response to lipid	25	7.34E-005
GO:1901700~response to oxygen-containing compound	33	8.10E-005
GO:0031328~positive regulation of cellular biosynthetic process	36	9.97E-005
GO:0014070~response to organic cyclic compound	25	1.97E-004
GO:0010033~response to organic substance	46	7.56E-004
GO:0051173~positive regulation of nitrogen compound metabolic process	34	1.84E-003
GO:0008219~cell death	36	2.12E-003
GO:0006954~inflammatory response	19	4.44E-003
GO:0010557~positive regulation of macromolecule biosynthetic process	31	6.23E-003

GO, Gene Ontology.

we needed to control for participant-level differences. To this end, we used a Bayesian hierarchical mixture model to account for both the variability within and between individuals.⁸ The model estimates the log fold change for each gene and calculates a probability of the gene being differentially expressed [$P(DE)$]. Significant differentially expressed genes were selected considering a 5% Type 1 error rate [i.e., $P(DE) > 0.95$] after Bonferroni correction. Gene Ontology enrichment was done for significantly upregulated and downregulated gene using DAVID.⁹ DAVID is a web-based application that ranks biologically relevant gene sets enriched in the gene list being interrogated using a modified Fisher exact test.

On average, the RNA-seq from the UC participants resulted in ~6.6 million uniquely aligning reads per sample. Differential expression analysis of this data resulted in a total of 324 significant differentially regulated genes, out of which 167 were downregulated and 157 upregulated post-AIP dietary intervention. The 10 highest scoring Gene Ontology terms for biological processes associated with the significantly upregulated and downregulated genes are presented in Table 2. Functional pathway analysis of differentially expressed genes indicated transcriptional changes associated with downregulation of inflammatory T-cell-mediated responses, as well as increased regulatory T-cell responses and function. On the other hand, we observed post-diet upregulation of transcriptional pathways associated with the inflammatory response and mucosal healing, including protein synthesis, fatty acid synthesis, and DNA repair.

Elimination diets are thought to act in various ways, by restoring balance of intestinal flora, modulating immune activation, reducing overgrowth of proinflammatory bacteria, and promoting nutrient intake and mucosal healing.^{3,10} Other than the AIP diet, other elimination diets studied as dietary therapy for patients with

active IBD include the specific carbohydrate diet, the CD exclusion diet with or without partial enteral nutrition, the low-fermentable oligosaccharide, disaccharide, monosaccharide, and polyol (FODMAP) diet, the IBD-anti-inflammatory diet, and the CD treatment-with-EATING diet, and have recently been summarized.¹¹ Most of these studies are uncontrolled, with elimination phases ranging from 2 to 12 weeks, and variable or undefined maintenance phases. Clinical response or remission rates among these diets range from 40% to 80%, but only a few examine changes in biomarkers, fecal microbiome, and/or mucosal healing.

We observed modulation of pathways involved in inflammation, DNA repair, metabolic processes, and cellular proliferation, suggesting cellular responses to dietary modification. Mucosal T-cell proliferation is generally upregulated in IBD along with a cross-talk between lamina propria lymphocytes and intestinal epithelial cells, which leads to an enhanced differentiation pattern of the intestinal epithelial cells in patients with IBD.^{12,13} Mucosal inflammation is also central to IBD and results in high cellular oxidative stress, which results in DNA damage, lipid peroxidation, and protein carbonyl formation. Furthermore, intestinal fatty acid metabolism has been previously shown to be decreased in IBD patients.¹⁴

Results from this RNA substudy would suggest dietary elimination, along with emphasis on a nutrient-dense diet, has the potential to positively modify inflammation and reduce symptoms in UC. Our substudy, though very small, included a well-defined subgroup of participants with UC on mesalamine only, avoiding effects that might be

attributable to systemic or targeted immunosuppression. Important limitations include a very small sample size and lack of control group, as well as potential confounding but non-diet-related factors, such as lifestyle, other medications or supplements, and natural history of the disease. Larger randomized controlled trials are needed to examine efficacy of dietary interventions on not only symptoms, but also endoscopic disease activity, and assess unique dietary aspects affecting inflammation in IBD.

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