Original Research

Treating Irritable Bowel Syndrome with a Food Elimination Diet Followed by Food Challenge and Probiotics

Jeanne Drisko, MD, CNS, Bette Bischoff, MD, RD, Matthew Hall, PhD, Richard McCallum, MD

Program in Integrative Medicine (J.D.), School of Medicine (B.B.), Preventive Medicine and Public Health (M.H.), Gastrointerology and Hepatology (R.M.), University of Kansas Medical Center, Kansas City, KS

Objective: In Irritable Bowel Syndrome, the gut-associated immune system may be up-regulated resulting in immune complex production, low-grade inflammation, loss of Class I bacteria, and translocation of inflammatory mediators and macromolecules outside of the GI lumen. Since food intolerance may be one of the reasons for this upregulation, our goal was to investigate the role of food intolerance in IBS patients.

Methods: In this open label pilot study, we enrolled 20 patients with IBS by Rome II criteria (15 women, ages 24–81) who had failed standard medical therapies in a tertiary care GI clinic. Baseline serum IgE and IgG food and mold panels, and comprehensive stool analysis (CSA) were performed. Breath-hydrogen testing and IBS Quality-of-Life (QOL) questionnaires were obtained. Patients underwent food elimination diets based on the results of food and mold panels followed by controlled food challenge. Probiotics were also introduced. Repeat testing was performed at 6-months. We followed up with this cohort at 1 year after trial completion to assess the reported intervention and for placebo effect.

Results: Baseline abnormalities were identified on serum IgG food and mold panels in 100% of the study subjects with significant improvement after food elimination and rotation diet ($p < 0.05$). Significant improvements were seen in stool frequency ($p < 0.05$), pain ($p < 0.05$), and IBS-QOL scores ($p < 0.0001$). Imbalances of beneficial flora and dysbiotic flora were identified in 100% of subjects by CSA. There was a trend to improvement of beneficial flora after treatment but no change in dysbiotic flora. The 1-year follow up demonstrated significant continued adherence to the food rotation diet (4.00 ± 1.45), minimal symptomatic problems with IBS (4.00 ± 1.17), and perception of control over IBS (4.15 ± 1.23). The continued use of probiotics was considered less helpful (3.40 ± 1.60).

Conclusion: These data demonstrate that identifying and appropriately addressing food sensitivity in IBS patients not previously responding to standard therapy results in a sustained clinical response and impacts on overall well being and quality of life in this challenging entity.

INTRODUCTION

Irritable Bowel Syndrome is the most common functional gastrointestinal disorder with a reported prevalence in the general population between 12%–22% [1–4]. In fact, IBS is the most common diagnosis made by gastroenterologists in the United States, accounting for 12% of visits to primary care providers [2]. IBS is a diagnosis of exclusion developed by a consensus definition and criteria known as the Rome II Criteria [5].

IBS is a disorder that is poorly understood with high direct and indirect associated medical costs [6,7]. Successful therapeutic options have been difficult to develop because of the lack of pharmacological targets and wide range of symptomatology [3,8,9]. As a result, an attempt is made to suppress symptoms with anti-cholinergic, anti-spasmodic, anti-diarrheal, and serotonergic agents with variable success as symptoms are not completely eliminated.

The gut is the largest lymphoid organ in the body [10]. In IBS, the gut-associated immune system is up regulated as evidenced by increased inflammatory cytokines such as interleukin 1, 6, and 10 [3,11–13]. The etiology of this altered

immunity is unclear but may be related to food hypersensitivity and/or altered GI microbial environment combined with altered enteric nervous system sensation. It is known that there is abnormal fermentation in IBS [14] and this leads to immune up-regulation [15]. It is also known that there is change in symbiotic, commensal, and dysbiotic microbial gut colonies in IBS [16–20]. It has been reported that types of microflora colonizing the gut play a role in regulating immunity [21]. In addition, upregulated GI associated immune tissue is known to stimulate discharge of enterochromaffin cells and other cells, which release serotonin and/or histamine resulting in GI symptoms [22–29]. Inflammation can result in opening of tight junctions between enterocytes with translocation of large proteins across the GI lumen. These proteins act as antigens systemically and antibody production results [21,22,30].

It is our hypothesis that correcting the luminal micro-environment will lead to improvements in IBS symptoms. This may be accomplished by a two-pronged approach. First, food and mold hypersensitivity [14,18,22,30–33] contributes to the altered inflammatory environment in the GI track and serum IgE and IgG food and mold panels can guide a food withdrawal diet, resulting in improved symptom complex [15,32–36]. If the results of the IgE and IgG food and mold panels are significant, subsequent systematic food challenges should result in IBS symptom recurrence. Secondly, altered microbial environments that are related in IBS may be corrected by probiotic administration [17,20,37–43].

METHODS

Study Design

The reported prospective outcome study with multifactorial intervention enrolled a cohort of diarrhea dominant irritable bowel syndrome patients from a tertiary care gastroenterology clinic. Prerequisite for entering into the study included a diagnosis of IBS by Rome II criteria and evaluation by gastroenterologists at the University of Kansas Medical Center. The following laboratory tests were required to be within normal range: total blood count, erythrocyte sedimentation rate, biochemistry screen, routine stool evaluation including culture, examination for occult blood, ova, and parasites, and a recent normal sigmoidoscopy or colonoscopy within 2 years of enrollment. Persons were excluded if organic intestinal disease was present. Subjects were also excluded if there had been recent antibiotic use or recent or concurrent enrollment into an IBS study.

Twenty-five subjects were screened between December 2001 and October 2002 and 20 were enrolled; of those enrolled, there were 5 men and 15 women consistent with national statistics [2,3]. See Table 1. Three patients declined to participate (refusal to obtain colonoscopy/sigmoidoscopy, or refusal to adhere to dietary requirements); one was excluded based on

Table 1. Summary of Demographic Data

	Male	Female
Number Enrolled	5	15
Age range (mean)	43 yrs–77 yrs (57)	24 yrs–80 yrs (49)
Duration of IBS Symptoms	3 yrs–50 yrs (22)	3 yrs–60 yrs (23)
Results of Breath Hydrogen at Baseline	2 positive	2 positive

definable organic pathology in the gastrointestinal tract (porphyria), and 1 did not fulfill the strict Rome II criteria. One of the study subjects withdrew from the study after 2 months stating refusal to adhere to dietary requirements. Data was analyzed on intent to treat basis.

Baseline requirements included a visit with the gastroenterologist, comprehensive IBS symptom and quality of life questionnaires (University of North Carolina School of Medicine—Chapel Hill GI Psychosocial Research Group), and hydrogen breath testing to assess for small bowel bacterial overgrowth. Subjects had 7 visits, including the baseline visit and 6 monthly intervention visits; after completion of the study, a follow-up visit with a gastroenterologist was required. At baseline, the serum IgE and IgG food and mold antigen panels (Allos Reference Laboratory—Hitachi Chemical Diagnostics Inc., Mountain View, CA) and stool collection for comprehensive digestive stool analysis were obtained (Great Plains Laboratory, Overland Park, KS).

Study subjects each received a tailored food withdrawal diet based on the results of the serum IgE and IgG food and mold antigen panels. The food and mold withdrawal diet was followed for 21–28 days with subsequent individual food challenges performed over several months. Food and symptom diaries were kept during the challenge phase and reviewed by the investigators. If a food was tolerated during the challenge phase, the food was reintroduced back into the diet with instructions to adhere to a rotation diet. If IBS symptoms returned with food challenge, the food was eliminated from the diet for 6 months with instructions to rechallenge at a later date. Study subjects were given probiotics (Vital 10 powder, ¼ teaspoon 2X/day, Klaire Labs, Solana Beach, CA) beginning at month 2 after the food and mold elimination diet period. The probiotics were taken daily from months 2 through 6 followed by a 1 month washout period.

At one year after trial completion, a follow up questionnaire to assess gastrointestinal status was obtained; this was to evaluate for the role of placebo effect in this intervention, which is known to be quite high in IBS [29].

The protocol was approved by the Investigational Review Board of the University of Kansas Medical Center. All participants provided written informed consent.

Breath Hydrogen and Methane Testing

The importance of breath testing is acknowledged [44]. All subjects presented to the GI Motility and Functional Bowel

Center at the University of Kansas Medical Center for the hydrogen and methane breath test after an 8-hour fast. Subjects ingested 30 cc of glucose syrup after a sample of their breath was collected at baseline. Further breath samples were obtained every 15 minutes for 3 hours. All breath samples were end-expiratory and analyzed immediately by model DP Microlyzer-chromatograph (Quintron Instrument Company, Milwaukee, WI). The concentration of breath hydrogen and methane was measured in parts per million (ppm). The measurements were plotted graphically and analyzed. The diagnosis of small intestine bacterial overgrowth was based on the following criteria: A peak within 90 minutes of hydrogen or methane concentration of > 20 ppm, or a baseline level of hydrogen or methane of > 10 ppm, which subsequently continued to increase after glucose challenge.

Comprehensive Digestive Stool Analysis

Each study subject collected stool samples for a comprehensive stool analysis (test kits provided by Great Plains Laboratory, Overland Park, Kansas and conducted by Doctor's Data, West Chicago, Illinois) on 2 consecutive days at baseline and again at the end of the intervention for comparison. Bacteriology, aerobic evaluated by BAP, Mac, CNA, and HEK plates to identify Salmonella, Shigella, yersinia, vibrio, and Aeromonas plus any other pathogenic bacteria. GN broth is used to isolate pathogens in smaller quantities and API for an identification system; results are reported in organism type and level from 0, 1+, 2+, 3+, 4+ with 0 representing no colonies and 4 the highest count. Bifidobacter is cultured on anaerobic culture media (modified CNA plates from Oxyrace create anaerobic environment); results reported in organism and level 0, 1+, 2+, 3+, 4+. Enterohemorrhagic E. Coli, Giardia, E. Histolytica, and Cryptosporidium are evaluated by EIA kit-ProSpect from Alexon Trend by Remel with results reported as positive or negative. Campylobacter is identified by EIA Kit-ProSpect from Alexon Trend by Remel and cultured with microphilic environment pouch on campy plates with results positive or negative. Parasitology identification Trichrome Stain, and concentrate are evaluated by microscopy and identified parasites reported. Yeast culture is expressed as definitive identification. Disc susceptibilities for yeast and bacteria are done by Kirby-Bauer and reported as sensitive or resistant. Cholesterol in the stool is identified by colormetric chemistry analyzer (Olympus AU600—Kit reagents used are from DCL); results are given in mg/dL. Chymotrypsin amount in the stool is determined by colormetric chemistry analyzer (see above); results are given in IU/g. Fecal Lactoferrin and Lysozyme are evaluated by Latex Agglutination with results positive or negative. Fecal secretory IgA is measured by EIA Kit from ALPCO; results expressed in ng/mL. Meat fibers, red and white blood cells are counted by direct microscopy and reported as none, few, moderate, or many. Occult blood is evaluated by guiac—Hemoccult and results reported as positive or negative.

The pH of the stool is measured by a pH meter and reported as a number from 0–14. Short Chain Fatty Acids in the stool to include acetate, propionate, butyrate, and valerate are measured by Gas Chromatography—Varian GC/MS and Acetate: expressed as percent of total of the total N-Butyrate expressed as μg/g. Steatocrit is measured by capillary microcentrifugation and reported in ng/mL. Triglycerides in the stool are measured by colormetric chemistry analyzer and reported in mg/dl. NEFA (Non-Estrified Fatty Acids) are identified by colormetric chemistry analyzer (off-label use of Wako kit for serum). Test results were reported within 4 weeks and collected at baseline and again at completion of the intervention.

Serum IgE and IgG Panels

Venous blood was collected in a 10 mL serum separator tube. Blood was allowed to stand in the serum separator tube for 20 minutes. The samples were centrifuged at 3,000. Samples were immediately placed cold packs and sent to the Allos Reference Laboratory (Hitachi Chemical Diagnostics, Inc., Mountain View, California). The test was performed at baseline and again at the end of the 6-month intervention, but only the IgG was repeated at the completion of the trial since it was assumed that the results of the IgE would not change in the time period.

For analysis, the serum was drawn into a sealed test chamber containing 36 threads coated with antigens specific for food and mold. (The test chamber contains a negative control and a positive control with IgG, for the IgG system, or IgE, for the IgE system, covalently bound). The test chamber with the serum was incubated at room temperature for 18–24 hours; the serum was drained from the holding chamber.

The test chamber was washed with wash buffer, drained, and filled with antibody reagent, and incubated at room temperature for 4 hours. After draining and washing, the photoreagent mix was drawn into the test chamber and incubated for 10 minutes. After 10-minute incubation, photoluminescence was measured and reported in luminescence units. Results are reported using a classification system based on relative light unit system. The luminescence units were reported as class values and assigned a numerical rating from 0–4 based on the amount of light admitted by the individual threads in the test chamber. Class values of one or greater represents progressively increasing concentrations of allergen specific antibodies. Class zero represents an absence or nondetectable levels of allergen specific antibodies.

The sensitivity detection limit of the assay is ten luminescence units. There is less than 1% cross reactivity with human serum immunoglobulins IgA, IgM, IgG, or IgD at normal physiologic levels. On average, concordance (calculated as efficiency) between CLA allergen and alternative in-vitro assay is approximately 95%; the range of concordance is 86%–100%. There are no standardized reference allergens available for

comparisons between methods, or for the great majority of clinical relevant allergens.

Probiotic Supplement

Replacement of beneficial microflora was by probiotic supplementation (Vital-10, Klaire Labs, Solana Beach, CA 92075). The product contained *Lactobacillus acidophilus, Bifidobacterium baifidum, L. rhamnosus, L plantarum, B. infantis, L. salivarius, L bulgaricus, L casei, L brevis,* and *Streptococcus thermophilus.* Each dose gave a total of 10+ billion colony forming units and was taken twice each day with meals to assist in adherence to the gut wall.

Outcome Variable: Irritable Bowel Quality of Life Outcome Instrument

Permission was obtained to use the IBS specific symptom diary and QOL instrument (University of North Carolina School of Medicine—Chapel Hill GI Psychosocial Research Group). IBS-QOL is a validated survey instrument [45,46]. The original version of the IBS-QOL contains 34 question items that ask about the patient's feelings and response is measured on a 5-point Likert-scale where 1 = not at all, 2 = slightly, 3 = moderately, 4 = quite a lot, 5 = a great deal/extremely. All items are summed-scored to calculate total scores (overall score). Subscales are collected for dysphoria, interference with activity, body image, health worry, food avoidance, social reaction, sexual, and relationship. The IBS-QOL was obtained in all study subjects as a baseline survey and at completion of this study.

Predictor Variables

A record of objective clinical findings of change in stool frequency and pain and subjective quality of life were obtained at baseline and at completion of the study. In addition, the changes in IgG food and mold scores from baseline to completion were measured after the treatment intervention. Finally, stool microflora counts were assessed at baseline and after probiotic use by the comprehensive stool analysis.

1-Year Follow Up Questionnaire

To evaluate for the role of the placebo effect in this intervention, a follow-up questionnaire was administered at 1-year post intervention. There had been no significant contact with the study subjects by the study team in the interval. All twenty patients were contacted and completed the questions. Four questions were asked to evaluate current IBS symptoms, adherence to a rotation diet, use of probiotics, and attitude about control over IBS symptoms. The questions were based on a 5-point Likert-scale ranging from 1 = strongly disagree and 5 = strongly agree.

Statistical Analysis

The goal of this study was to examine the contribution of serum IgG food and mold antigen levels for tailoring food and mold withdrawal/rotation diets and its impact on IBS symptoms and QOL. In addition, it was necessary to assess the importance of stool microflora colonies and the impact of probiotic products on IBS symptoms and QOL scores. We first summarized all measurements with their means and standard deviations. Wilcoxon's Signed Rank Test was applied to determine if there is a significant change in each measure from baseline to completion.

RESULTS

Patient Characteristics

Of the 20 patients enrolled in the study, 5 were male and 15 were female. The age range for the men was 43–77 (57) years and for the women 24–80 (49) years. The duration of IBS symptoms for the men was 3–50 (22) years and for the women was 3–60 (23) years. There were 2 positive breath hydrogen tests at baseline in each group of men and women, which correlated with abnormalities on comprehensive digestive stool analysis. See Table 1.

Pain and Stool Frequency

In this prospective study in a cohort of diarrhea dominant IBS subjects, systematic food withdrawal guided by the results of the IgG and IgE food and mold panels resulted in significant improvement in symptoms including stool frequency and severity of pain. At baseline reported stool frequency was 4.29 (2.49) stools per day and at completion were 3.43 ($\pm$ 1.22) stools per day (P < .05). Pain diary scores based on a pain scale from 1 (none) to 5 (most severe) resulted in a significant improvement from baseline of 3.65 ($\pm$ 1.12) to completion of 2.71 ($\pm$ 1.38) (P < .05). See Table 2.

IBS QOL

Response was measured on a 5-point Likert-scale where 1 = not at all to 5 = a great deal/extremely with all items summed-scored to calculate total overall score from baseline to completion. In final data analysis, the five responses are transformed in order to obtain a 100-point overall QOL score and eight 100-point subscales. After conversion, higher scores denote a higher quality of life and lesser degree of IBS symptoms. This cohort demonstrated a significant improvement in overall QOL scores [46.51 ($\pm$ 21.08) to 67.22 ($\pm$ 20.92); P < .001]. Subscales collected at baseline and completion for dysphoria [37.66 ($\pm$ 23.64) to 66.28 ($\pm$ 24.58); P < .001], interference with activity [40.54 ($\pm$ 21.81) to 65.23 ($\pm$ 24.60); P < .001], body image [59.69 ($\pm$ 23.52) to 76.32 ($\pm$ 18.47); P < .001], health worry [58.33 ($\pm$ 24.63) to 77.63 ($\pm$ 20.42); P = .002], food

Table 2. Results

	Baseline Mean (Std)	Completion Mean (Std)	Signed Rank P-Value
# of Stools/day	4.29 (2.49)	3.43 (1.22)	<0.05
Pain Scale 1 (none)–5 (most severe)	3.65 (1.12)	2.71 (1.38)	<0.05
IgG Food Positive #	10.05 (10.08)	6.47 (8.85)	<0.01
IgG Food High Positive #	0.10 (0.31)	0.71 (2.26)	0.500
IgG Mold Positive #	3.30 (1.26)	2.63 (1.42)	<0.05
IgG Mold High Positive #	1.35 (1.69)	1.79 (1.87)	0.069
IBS-QOL			
Dysphoria	37.66 (23.64)	66.28 (24.58)	<0.001*
Interference w/Activity	40.54 (21.81)	65.23 (24.60)	<0.001*
Body Image	59.69 (23.52)	76.32 (18.47)	<0.001*
Health Worry	58.33 (24.63)	77.63 (20.42)	0.002*
Food Avoidance	30.42 (26.80)	38.16 (25.36)	0.362
Social Reaction	48.13 (32.64)	69.08 (24.07)	0.002*
Sexual	73.13 (27.89)	79.61 (29.82)	0.100
Relationships	55.00 (32.83)	70.18 (25.36)	<0.001*
Overall	46.51 (21.08)	67.22 (20.92)	<0.001*
Beneficial Bacteria (Class I)	2.07 (1.54)	2.67 (1.30)	0.250
Dysbiotosis/Parasitology	1.58 (0.84)	1.47 (0.91)	0.620

* Significant at the 0.0025 level.

avoidance [30.42 ($\pm$ 26.80) to 38.16 ($\pm$ 25.36); P = .36], social reaction [48.13 ($\pm$ 32.64) to 69.08 ($\pm$ 24.07); P < .002], sexual [73.13 ($\pm$ 27.89) to 79.61 ($\pm$ 29.82); P = .10], and relationships [55.00 ($\pm$ 32.83) to 70.18 ($\pm$ 25.36); P < .001]. See Table 2.

Serum IgG Food and Mold Antigens

Baseline serum IgG food reactions were measured in luminescence units (LU) with the range from 0–11 = negative, 12–26 = equivocal, 27–65 = low positive, 66–142 = positive, and 143 $\geq$ 242 = high positive. For the purpose of the food and mold elimination diet, only reactions that were positive to high positive were considered. At baseline, there were 10.05 ($\pm$ 10.08) positive IgG food reactions identified per patient and at completion after food elimination, 6.47 ($\pm$ 8.85) P < 0.01. At baseline, there were 0.10 ($\pm$ 0.31) high positive IgG food reactions and at completion 0.71 ($\pm$ 2.26), which did not show a significant change. At baseline, there were 3.30 ($\pm$ 1.26) positive IgG mold reactions identified and after completion a reduction to 2.63 ($\pm$ 1.42) P < 0.05. At baseline there were 1.35 ($\pm$ 1.69) high positive IgG mold reactions and at completion 1.79, which was not significant.

The most frequent positive serologic IgG antigen-antibody complexes found on the food and mold tests were: 4 or more molds, 14 out of 20 patients (70%); baker's yeast, 17 out of 20 (85%); onion mix, 13 out of 20 (65%); pork, 12 out of 20 (60%); peanut 12 out of 20 (60%); corn, 11 out of 20 (55%); wheat, 10 out of 20 (50%); soybean, 10 (50%); carrot, 9 out of 20 (45%); cheddar cheese, 8 out of 20 (40%); egg white, 8 out of 20 (40%). See Table 3. Only 5 out of 20 reacted by IgG antibody production to dairy; however the majority of patients reported eliminating dairy prior to trial enrollment presumably clearing antigen-antibody complexes prior to testing.

Microflora Colony Counts

In the comprehensive digestive stool analysis, colony counts of microflora are expressed as a range of 0 to 4+ colony counts with 0 being no colonies identified and 4+ as the maximal colony count. At baseline prior to probiotic intervention, the study subjects were found to have a trend to improvement in Class 1 beneficial microflora at 2.07 ($\pm$ 1.54) colony counts and after probiotic supplementation, beneficial colony counts rose to 2.67 ($\pm$ 1.30). Counts in dysbiotic microflora at baseline were found to be (1.58 ($\pm$ 0.84) and found not to clear with probiotic replacement from 1.58 ($\pm$ 0.84) to 1.47 ($\pm$ 0.91).

1-Year Follow-up Questionnaire after Trial Completion

One-year follow-up questionnaire had 4 questions with responses based on a 5-point Likert-scale ranging from 1 =

Table 3. Most Frequent Positive Serologic IgG Antigen-Antibody Food and Mold Tests

4 or more molds	14	70%
Baker's yeast	17	85%
Onion mix	13	65%
Pork	12	60%
Peanut	12	60%
Corn	11	55%
Wheat	10	50%
Soybean	10	50%
Carrot	9	45%
Cheddar Cheese	8	40%
Egg White	8	40%
Milk (dairy)*	5*	25%*

* Dairy is often considered to be one of the foods that should be eliminated. However, this cohort did not show a high prevalence of IgG Ag-Ab complexes. It may be that many of these subjects had already eliminated dairy and cleared dairy specific IgG Ag-Ab complexes.

strongly disagree and 5 = strongly agree. All 20 study subjects responded. The questions included adherence to the food rotation diet (4.00 ± 1.45), minimal symptomatic problems with IBS (4.00 ± 1.17), and perception of control over IBS (4.15 ± 1.23). The ongoing use of probiotics at 1-year was found to be less helpful (3.40 ± 1.60).

DISCUSSION

The reported multifactorial intervention resulted in significant improvements in irritable bowel symptom complex and QOL in this cohort of IBS diarrhea dominant patients. The patients enrolled in this study were on whole a difficult group of patients to manage having found their way to the tertiary care gastroenterology clinic. They were not felt to be adequately improved by extensive use of standard medical therapy and care given by a single gastroenterologist with expertise in this area (RM). Identifying and appropriately addressing food hypersensitivity and abnormal bowel microenvironment in IBS patients not previously responding to standard therapy resulted in a significant clinical response. This improvement was found to be sustained at 1 year post-intervention during which time there was no significant contact with the investigators; the 1-year follow-up was done to evaluate the role of the placebo effect as a major factor in the improvement. The improvement was found to be both objective with reduction in pain and diarrhea as well as subjective with increased quality of life. The majority of study subjects continued to adhere to the rotation food diet at 1 year post intervention and felt they had reduced symptoms and increased control over their IBS.

The gastrointestinal tract is the largest immune organ and responsible for vigilance and surveillance of ingested materials. Up-regulation of gut immunity, resulting in increases in inflammatory cytokines and other inflammatory mediators, is associated with IBS [3,10–13]. To date, it is unclear specifically what causes the immune stimulation in IBS and since IBS is a complex chronic disorder, there may be several contributing factors that lead to the change in immunity. Abnormalities in microbial biomass with decrease in Class 1 symbiotic microflora and increase in dysbiotic flora will cause changes in the patterns of immunity as do food and mold related hypersensitivity that results in increased immune complex formation.

Immunoglobulin G (IgG) may be a helpful marker of immune response for food hypersensitivity and delayed food reactions [33]. In a blinded trial, Atkinson and colleagues reported a benefit in IBS symptoms when evaluating IgG food withdrawal diets when compared to sham diets. The circulating elevated IgG may or may not be the cause of the symptoms but its ability to form immune complexes with antigens and to activate complement certainly fulfills the condition for immunopathologically-mediated inflammation [47]. Activation of immune reactions by non-IgE immune complexes that result

from delayed hypersensitivity may explain many of the observed reactions to food such as asthma, migraines, headaches, arthritis, gastrointestinal dysfunction, etc [48]. By focusing solely on IgE mediated reactions and excluding other elements of the immune response, important etiologies of patients' symptoms are overlooked.

The parallel assays of specific IgG and IgE antibodies to food and mold provide an approach to determining offending foods in the clinical situation. After antibodies to specific foods are detected, the patient is placed on a food elimination diet for two to four weeks, after which foods that do not mediate IgE reactions are systematically returned to the diet one at a time [33,48–50]. Patients with true food hypersensitivities should have clear reactions with food challenges, but these reactions may not occur until hours or days post ingestion. Detailed food diaries are necessary during the challenge phase to assess for delayed hypersensitivity reactions. Open food challenges are usually accurate and sensitive for testing non-IgE mediated food reactions in clinical practice and placebo effect is generally not a problem [48].

It should be noted that IgG food testing has not been considered a particularly useful test by the general medical community [47,53–55]. It is believed that IgG is formed universally after the ingestion of food; IgG is generally considered to be a protective antigen and as a result the test is thought to be non-specific. This conclusion is now being challenged and re-evaluated [47]. IgG by itself may or may not be the mediator of the symptoms, but it's presence in measurable quantities may serve as an indicator that a protective antibody is necessary.

The rationale for adding IgG testing is based on the findings that certain subclasses of IgG or non-IgE associated reactions have been associated with *in vitro* degranulation of basophils and mast cells, the activation of complement cascade, and the observation that high circulating serum concentrations of some IgG have been measured in certain atopic individuals [47,48,56]. Based on the results of the IgE and IgG mold panels, an appropriate food elimination diet may be implemented. It has been shown that decreased lymphocyte proliferation responses, improved clinical outcomes, and decreased release of inflammatory mediators followed the tailored food elimination diet [36,48,49].

Food elimination diets and food challenges are extremely time consuming for the patient and practitioner and the elimination/challenge diet requires a high degree of patient motivation and compliance [33,35,48]. Although the serum IgE and IgG testing may help guide the food elimination diet initially, the oral food challenge remains the only modality to identify a true clinical reaction [48,51,52]. After the food challenge phase is complete and the offending foods are identified, these foods may be added back into the diet on a rotation basis of not more than 3 to 4 days between ingestion. That is, no food may be eaten repeatedly on successive days because antigen—antibody complexes may again accumulate, which result in recurrent symptoms of IBS or food intolerance. In a rotation diet for

example, wheat (gliadin or gluten antigen) sensitive patient may eat wheat containing foods once every 3 to 4 days with no wheat products consumed in between those days. Some patients find that if their initial reaction after challenge is severe, certain food groups may never be tolerated again in the diet without provoking symptoms. In any event, teaching patients to evaluate symptoms, correlate symptoms with food diaries, and manage specific food withdrawal and rotation diets gives them some measure of control over their functional bowel complaints.

Caveats regarding IgG food testing include a lack of intra-laboratory reproducibility, skepticism concerning the role of IgG food related antibodies in the pathophysiology of adverse reactions to food, the possibility that many adverse reactions to foods are pharmacologically or contaminant mediated and not detectable through immunological assays, and the possibility that digestion alters the protein make up and therefore its allergenicity. Another valid concern is it is not known what percentage of the population free of bowel symptoms has elevated IgG food-related antibodies.

Recently, O'Mahony and colleagues (2005) demonstrated improvements in IBS symptoms in a blinded trial with the addition of *Bifidobacterium infantis* 35624 in the diet with normalization of the ratio of anti-inflammatory to proinflammatory cytokines. These investigators did not find a similar effect when *Lactobacillus salivarius* UCC4331 was added. In other clinical trials, *Lactobacillus plantarum* 299v and DSM 9843 strains were shown to reduce abdominal pain, bloating, flatulence, and constipation [17,57]. It was also observed that *Saccharomyces boulardii* decreased only functional diarrhea in irritable bowel syndrome but was not effective in alleviating other symptoms of the syndrome [58]. In this trial, the reported improvements by probiotics on symptoms may be related to specific physiological effects of altered cytokine production, microflora cross-talk, or other direct effects and should be considered in an expanded evaluation. It was beyond the scope of this study to test for the direct effects of probiotics on the gastrointestinal tract.

At baseline, the cohort of patients in this study demonstrated decreased Class 1 beneficial microflora with decreased colonies of *Lactobacillus sp., bifidobacteria sp.*, and beneficial *E-Coli.* In addition, there were increased dysbiotic microflora colonies and fungal species in a subset of patients with positive breath testing. Of note, there was a trend to improvement in the Class 1 microflora with probiotic supplementation over the course of the trial but this was not significant. This may be related to underestimating the amount of probiotic supplement necessary, type of flora necessary, or the duration of time needed to effect such a change [20,37,40]. It would be useful in the future to evaluate the dose response of various preparations with various colony counts and correlate this with the changes in colony counts on follow up stool testing and changes in gut immunity.

Although there was not a significant improvement in beneficial colony counts in this study, patients reported symptom relief when using probiotics. However at the 1-year follow up,

the study subjects were only sporadically continuing to use probiotic supplements. As stated, more aggressive replacement may be warranted or investigation into the direct effects of probiotics on immunity would be helpful.

Interestingly, probiotics alone were not sufficient to eradicate dysbiotic flora or produce normalization in follow-up breath-hydrogen testing in the patients that had positive breath-hydrogen tests at baseline. After the trial was complete and not at baseline, antibiotics, with documented sensitivities to the abnormal flora, were given to eradicate the dysbiosis in this group of patients. It should be noted in this subset of patients, the majority of IBS symptoms improved prior to dysbiotic flora eradication. Since the sample size was small, this cannot be commented on further but should guide further investigation when enrolling subjects who have positive breath-hydrogen tests and dysbiotic flora found on stool testing.

The patients enrolled in this trial demonstrated significant improvements in quality of life (QOL) assessment. Ultimately, improving QOL for the functional bowel patient is the most important benefit. QOL is a term that has been used to denote outcomes as experienced by the patient and there has been growing interest in the use of health-related quality of life measures in gastrointestinal disorders. QOL measures in clinically ill individuals encompasses multiple domains or areas of well-being (including, at a minimum, physical, psychological and social functioning, as well as symptoms) and the perspective of the patient is critical in any measurement of QOL. Functional bowel disorders have been studied pre- and post-treatment with health status outcome measures [45,46,59–61]. The validity of using the outcome instruments has been documented and has been a useful tool for following the therapeutic benefits of treatment in clinical practice and in controlled trials. While patient perspectives are important in any health condition, they become particularly so in diseases that are chronic such as IBS.

The reported trial is small and the food challenges were open and not blinded, although some believe that reactions related to IgG delayed hypersensitivity and tracked over 72 hours after the food challenge can safely be attributed to that specific food [48], however a repeat larger trial with blinding is warranted. In addition, the sensitivity and specificity of IgG food testing needs to be evaluated and labs need quality controls instituted to assure reproducibility. Further trials with blinded food challenges may be necessary to overcome the bias against IgG food hypersensitivity testing. In addition, comparison to normal controls would be helpful to assess the significance of the IgG food related immune complexes. Furthermore, IgG is only a subsection of the immunity and represents only a small percentage of food hypersensitivity and there may be other causes of food hypersensitivity or increased inflammation in the GI tract besides the antigen/antibody complex formation. What may be even more helpful in further studies would be a more direct assessment of the bowel milieu after food challenge for changes in inflammatory cytokines and other immune messengers like histamine. This of course, by necessity, would

need to include assessment of neurotransmitter production in the gut such as serotonin excretion by the enterochromaffin cells. Other important questions to be answered would be the role of microflora cross-talk and the interaction of microflora populations on bowel immune messages.

As a result of the high numbers of the population affected by IBS, if simple solutions such as food elimination and rotation diets and maximizing bowel flora could result in significant improvements then potential savings to the health care system could result. IgG food testing would need to be validated with intra-lab reproducibility and then the extended healthcare team could manage the follow up diet manipulation and recommendations. This could reduce the time and expense of physician involvement and the use of expensive pharmacologic interventions. It is recommended that repeat evaluations include cost analysis studies.

The complex role of abnormal bowel flora coupled with the presence of food hypersensitivities on symptom production has been noted in a previous clinical trial of IBS [18]. Since both microflora and ingested materials display a direct effect on immunity, it is not surprising that both would play a complicated intertwined role in the production of symptoms in IBS. It is difficult to determine at the present time if the food intolerance precedes the altered gut microbial environment or if the reverse is true. However, it may be neither but rather both abnormalities contributing to the outcome of increased inflammation and up-regulation of the immune system, altered enteric neurotransmitter output, and abnormal fermentation, which all results in gas, pain, bloating, and diarrhea. Irritable bowel syndrome is a complex chronic disorder necessitating complex interventions.

ACKNOWLEDGMENTS

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