



USE OF THE LEAP MEDIATOR RELEASE TEST TO IDENTIFY NON-IGE MEDIATED IMMUNOLOGIC FOOD REACTIONS THAT TRIGGER DIARRHEA PREDOMINANT IBS SYMPTOMS RESULTS IN MARKED IMPROVEMENT OF SYMPTOMS THROUGH USE OF AN ELIMINATION DIET

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BACKGROUND

Diarrhea predominant IBS (D-IBS) is a common condition that is often refractory to standard therapy. Though some treatments may improve certain symptoms, there is no treatment that has been shown to result in improvement of global D-IBS symptoms. The Lifestyle Eating and Performance Mediator Release Test (LEAP MRT) is an *in vitro* test that detects non-IgE mediated food reactions that can trigger D-IBS symptoms. We report on our early experience with this dietary modification program.

METHODS

Ten patients who met Rome II criteria for D-IBS are reported in this study. These patients presented to our community-based gastroenterology practice and were evaluated by a gastroenterologist. Evaluation for other causes of their symptoms was based on the patient's previous evaluation and the discretion of the gastroenterologist. Typically, if not already employed in the past, a trial of standard therapy such as fiber and anti-spasmodic agents was attempted. If the patient didn't improve, they were then offered LEAP MRT testing. Using an *in vitro* assay, the patient's blood was tested for non-IgE mediated reactivity to 150 foods and food additives. A specific elimination diet that omitted the reactive foods was then designed for the patient. A Symptom Survey was employed to follow the patients for improvement in D-IBS as well as systemic symptoms. The survey graded multiple GI and systemic symptoms on a scale of 0-4 with increasing severity represented by a higher number. The maximum points possible for the entire survey was 236 and for the GI portion was 36.

RESULTS

Prior to beginning the LEAP MRT based elimination diet, the average score for the entire survey was 56.9 and for the GI portion was 19.1. After at least one month on the diet, the average scores had decreased to 26.3 and 6.3 respectively. Patients generally reported a marked improvement in their D-IBS symptoms, decreased systemic symptoms, and an overall increase in their feeling of well-being.

CONCLUSION

The LEAP MRT identifies non-IgE mediated immunologic food reactions that trigger D-IBS symptoms. Elimination of these foods from the diet results in a marked improvement in D-IBS and other systemic symptoms.

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INTRODUCTION

Diarrhea predominant IBS (D-IBS) is a common condition that is frequently encountered by primary care physicians and gastroenterologists. Typically, treatment has involved reassurance, use of anti-diarrheal and anti-spasmodic medications and general instructions to eat a healthy diet and avoid foods that may worsen the D-IBS symptoms. In some patients, this therapeutic approach can be effective. However, in the majority of patients, it has only a minimal effect on the typical GI symptoms of diarrhea, bloating, cramping and pain. In addition, this treatment usually has no effect on the other systemic symptoms that are often associated with D-IBS, including but not limited to various constitutional symptoms and common comorbidities.

We propose that this approach is generally ineffective in treating D-IBS because it does not address the fact that D-IBS appears to be an immunologically mediated disease in the sense that “loss of oral tolerance” and low level persistent inflammatory reactions characterize this population. A number of properly designed studies over the last several years have demonstrated that D-IBS is characterized by several distinct types of abnormal inflammatory changes in the small bowel and the large bowel which vary by whether the patient has a history of developing IBS after an enteric infection or not (PI-IBS vs. Non PI-IBS.)

Considered in the context of specific studies of the oral tolerance mechanisms, these changes are characteristic of a process described variously as non-IgE mediated delayed hypersensitivity reactions to typically benign dietary substances, or “Loss of Oral Tolerance.” The gut mucosal immune system is a complex system that must react to intestinal pathogens and other orally ingested toxic compounds and prevent them from penetrating the gut mucosa and gaining entry into the systemic circulation. It must work together with specific circulating immunocytes to adapt to the presence of benign antigens in a fashion which will dampen the normal inflammatory response to antigens which is an integral part of digestion taking place in the upper bowel.

The adaptive immune system must recognize harmless foodstuffs as benign oral antigens and not react to them, or blunt the normal reactions to antigen as the case may be. This process is termed “oral tolerance” and has recently been described in detail as to the immunocytes involved (from mast cells to T Cells). The loss of oral tolerance to specific dietary antigens can result in non-IgE mediated reactions (T Cells, neutrophils, and other classes) which lead to the release of cytokines and other pro-inflammatory mediators in the gut mucosa and the systemic circulation. This in turn can lead to the occurrence of the typical local and systemic symptoms seen in D-IBS.

The Lifestyle Eating and Performance Program (LEAP) is a patient-specific oligoantigenic diet program which is

uniquely effective for the patient suffering IBS symptoms with a diarrhetic component. The diet is based upon the results of the Mediator Release Test (MRT) offered by Signet Diagnostic Corporation. MRT is a patented, “common end point” in vitro assay which can detect the common end point of any abnormal response of circulating immunocytes to benign antigen challenge. As the common end point of such reactions is the synthesis and/or extracellular release of mediators such as proinflammatory cytokines, unlike single-point antigen challenge assays (IgG[x] or specific mediator assays) MRT can detect any such aberrant reaction irrespective of which single or multiple non IgE pathway is active or any single or multiple class of immunocyte response through a novel patented method of in vitro assessment of immunocyte response to antigen challenge.

In overview, a patient’s (untreated) whole blood sample is divided into 150 aliquots plus control samples. Each sample is incubated with a precise and specific dilution of pure extract of a specific food or food additive based upon the dietary habits of the population in question (different countries demonstrate unique eating patterns). Each is checked by the MRT device for the specific signs of cell mediated reactivity to the antigen challenged (imminent or actual mediator release).

Any type of cell mediated reaction to antigen, depending upon the dose-time relationships, causes imminent or actual release of intracellular mediators to the extracellular compartment. In a closed system, wherein the total volume is held constant comparing antigen challenged blood to unchallenged control samples, volumetric changes between the intracellular and extracellular compartments (expressed as Plasma Volume Differential or PVD) in normal asymptomatic subjects with intact oral tolerance are nominal in response to the antigen challenge. In symptomatic patients who have lost tolerance to certain antigens a significant change in the volume of intracellular versus extracellular mass can be detected post in vitro challenge. The immune response is not adequately blunted. Commencement of mediator synthesis, or actual release, is reliably detected. From this, accurate patient dietary therapy can be instituted.

Since the total volume of the aliquot is held constant, the absolute change in cell volume with extract exposure can be quantified (See Figure 1).

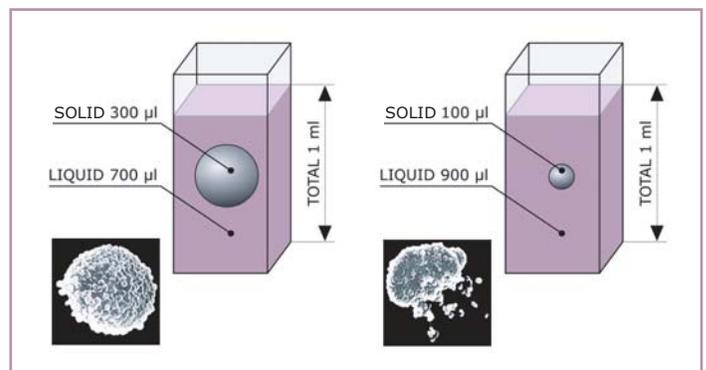


Figure 1

CLINICAL STUDY DESIGN

Since the instrument measures any change in total cell volume, it will detect any type of cell mediated reactivity and not just a single given cytokine or mediator. Thus overcoming the weakness of any single-mediator or single-pathway assessment since it has been shown that any of several, or multiple, types of reactions can occur in these patients. The common end point approach of MRT makes the LEAP Plan the most patient specific and comprehensive diet plan possible. Because immune reactivity is assessed by quantifying plasma volume differential, the relative degree of reactivity to a given food extract can also be quantified. Foods are recorded as Reactive, Moderately Reactive and Non-Reactive (See Figure 2). The more reactive the subject is to an antigen, the less dose-dependent and delayed onset the reaction is likely to be in vivo.

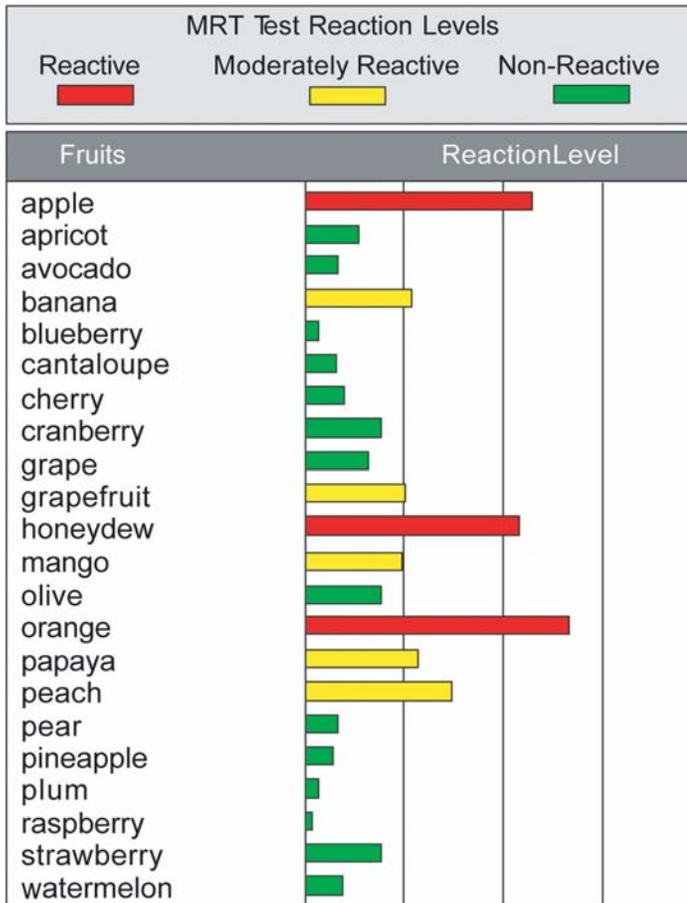


Figure 2

LEAP is unique in that the physician is now able to initiate treatment based on a selection of the least-reactive foods first, speeding clinical response to treatment and gaining credibility with the patient. Studies have shown this is vital to compliance. After one week, based on the remission of symptoms, the patient is then allowed to add other non-reactive foods based on the PVD results. In this novel way not only are any hidden actual food allergies quickly uncovered or dose dependent responses isolated, it provides a simple and logical structure to the plan which enhances compliance as well as speed and degree of relief. Patients will completely avoid all Moderately Reactive (yellow) and Reactive (red) foods.

The preliminary study consisted of ten patients who were referred to a community-based GI practice for (D-IBS) by their primary care physician. They were evaluated for possible causes of their symptoms as deemed appropriate by the Gastroenterologist in accordance with current clinical practice guidelines.

Typically, they were first treated with standard therapy such as anti-spasmodic, and anti-diarrheal medications. Patients who were non-responders to standard therapy and who met Rome II criteria for D-IBS were offered the option of treatment via the LEAP Program, which entailed the prerequisite MRT testing followed by the dietary modification program.

The patient's blood was drawn using standardized collection materials provided by Signet Diagnostic Corporation's clinical laboratory, and shipped overnight by FedEx to their CLIA licensed laboratory in West Palm Beach, Florida, where MRT testing was done and the LEAP plan for each patient compiled and then sent back to our facility for implementation. The patient then met initially for approximately one hour with one of our two nurse practitioners who had been trained by Signet in the LEAP protocol. They educated the patient on appropriate implementation of the LEAP dietary modification program.

Patients were seen for follow-up by the nurse practitioners at intervals of every two to four weeks during the first few months as determined by patient response. These sessions were for caregiver support, answering questions, and monitoring compliance and response to treatment. At the date of this presentation, the time on the diet for a given patient ranged from one to six months.

Prior to beginning the diet and at each follow up visit, patients were asked to rate symptoms in the following general categories on a scale of 0-4 with 4 being the most severe: gastrointestinal, constitutional, psychological, HEENT, skin, cardiopulmonary, musculoskeletal, genitourinary, or any weight changes. Specifically, they were asked to rate the following GI symptoms: heartburn, cramping, diarrhea, constipation, bloating, gas, nausea, vomiting, and painful elimination. The data presented for each patient is that of their initial visit before starting the LEAP MRT dietary modification program and their most recent visit.

RESULTS

Prior to starting the LEAP MRT dietary modification, patients had a mean score of 57 on a self-reporting symptom survey which takes into account both GI and non-GI symptomology commonly seen in IBS-D subjects. At the last time point available, this had decreased to a mean of 19. (See Figure 3)

Reduction of Global Symptoms with LEAP MRT Diet

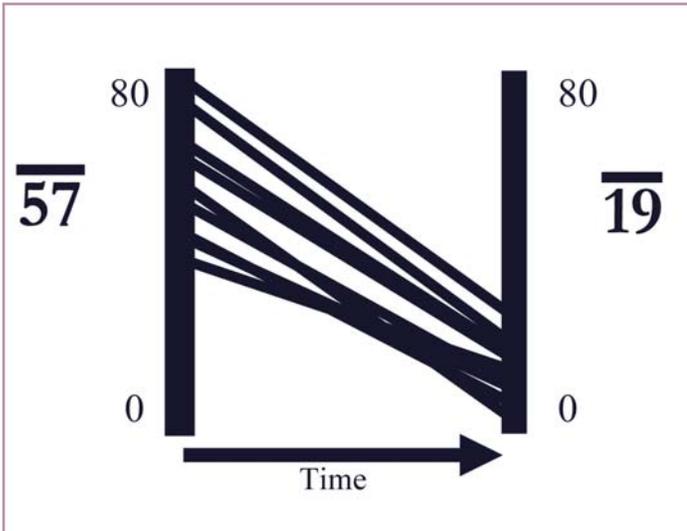


Figure 3

Prior to starting the program, patients had a mean gastrointestinal score of 26. At the last time point available, this had decreased to a mean of 6 (See Figure 4).

Reduction of Gastrointestinal Symptoms with LEAP MRT Diet

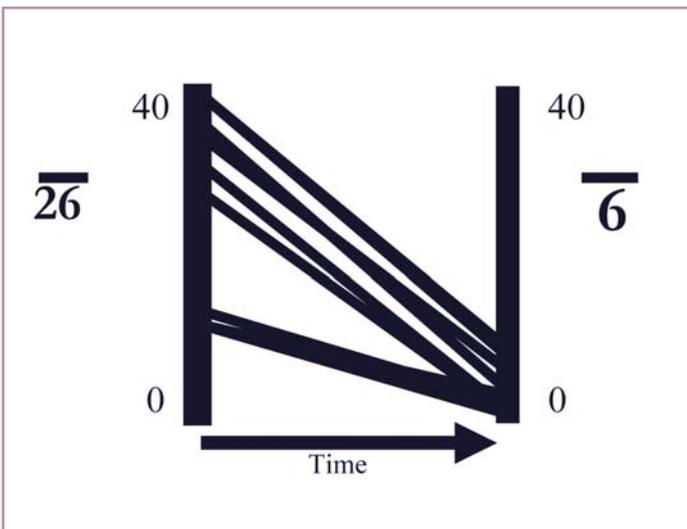


Figure 4

Overall, subjectively patients reported a general improvement in their feeling of well being and a decrease in both systemic and GI related symptoms. Many reported that they felt the best they had in years.

CONCLUSION

In conclusion, we report on ten patients with D-IBS who were refractory to standard therapy. They were placed on the LEAP MRT dietary modification program and had a marked improvement in their GI and systemic symptoms. We believe that this study supports the hypothesis that a substantial portion of D-IBS symptoms are due to loss of oral tolerance to typically benign oral antigens with subsequent development of non-IgE mediated delayed hypersensitivity reactions that result in release of various pro-inflammatory mediators which lead to the typical GI and systemic symptoms associated with D-IBS. Elimination of these offending nutritional antigens from the diet, can result in substantial improvement in D-IBS symptoms.

POST STUDY FOLLOW UP: CYTOKINES AS MEDIATORS IN D-IBS

During the time of this preliminary clinical assessment of LEAP treatment for D-IBS, Signet Diagnostics laboratory personnel were concurrently investigating potential additional QC procedures for Mediator Release testing. In particular Signet was interested in the quantitative analysis of plasma cytokines as a physiologic confirmatory method of MRT results and LEAP outcomes in IBS subjects. Prior published studies using novel in vivo jejunal isolation and antigen challenge methods, which included immunochemistry analysis of jejunal washings, had implicated proinflammatory cytokines as cellular mediators of the reactions seen in lost oral tolerance of D-IBS.

A preliminary trial consisted of a patient with a confirmed lifelong history (40 years) of Non-PI D-IBS who had been in remission for over one year on an MRT based LEAP diet. Analysis of plasma cytokine levels during his asymptomatic state was performed using a Bio-Rad Bio-Plex Dual Laser Flow Cytometer and Bio-Plex Protein Array System for Human Cytokines. The subject then began a 3-day open antigen challenge by taking him off his LEAP diet. He was on a "new diet" which included the test positive foods shown on MRT analysis of the subject's whole blood.

Within 72 hours the patient's GI and systemic IBS-D symptoms returned and he experienced a fulminating episode of severe IBS-D symptoms. Blood samples were again obtained, and the cytokine levels rechecked. The results are shown in the chart below. Plasma levels of several of the 16 proinflammatory cytokines assayed were markedly increased. This event corresponded to the reappearance of the patient's IBS symptoms. A familiarity with these cytokines and their respective physiologic functions sheds some new light on the mechanisms of symptom generation in IBS, and the physiologic basis for the efficacy of oligoantigenic diet therapy.

IBS-D patient plasma cytokines during d-episode v between episodes

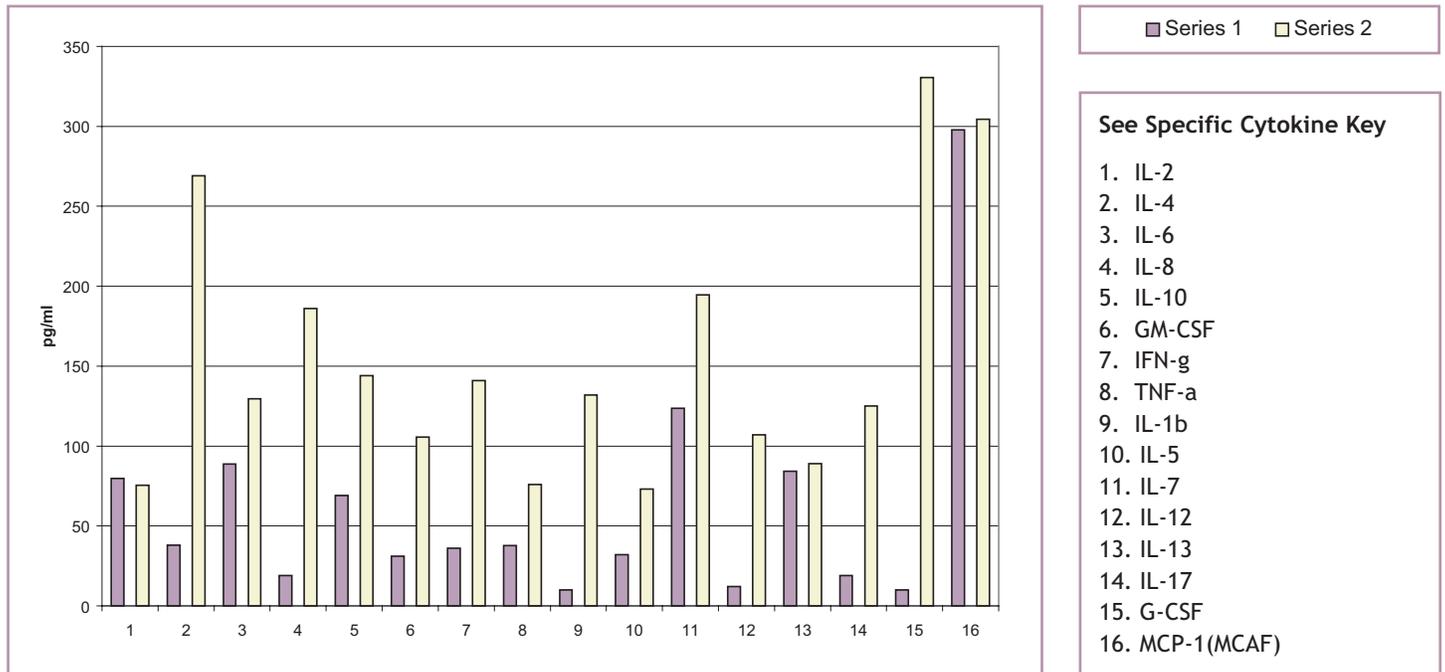


Figure 5

To quickly determine whether this result was unique to this patient or a possible misdiagnosis, or whether this might be a consistent pattern among D-IBS patients, Signet expanded the experiment and added a comparison to asymptomatic normal subjects. A group of asymptomatic normal subjects consented to plasma cytokine and MRT analysis. MRT results were unremarkable across the board, reflecting normal oral tolerance to the antigens tested and low residual levels of plasma cytokines were observed.

A group of patients was selected at random by physicians submitting blood samples from their symptomatic IBS patients for MRT analysis in anticipation of instituting the LEAP Program for these patients. An aliquot of plasma was also obtained, and frozen within 30 minutes of the sample being obtained, then both were sent to Signet for MRT analysis and cytokine analysis. The surprising results are shown in the chart below.

Plasma Cytokines: IBS-D vs Normals

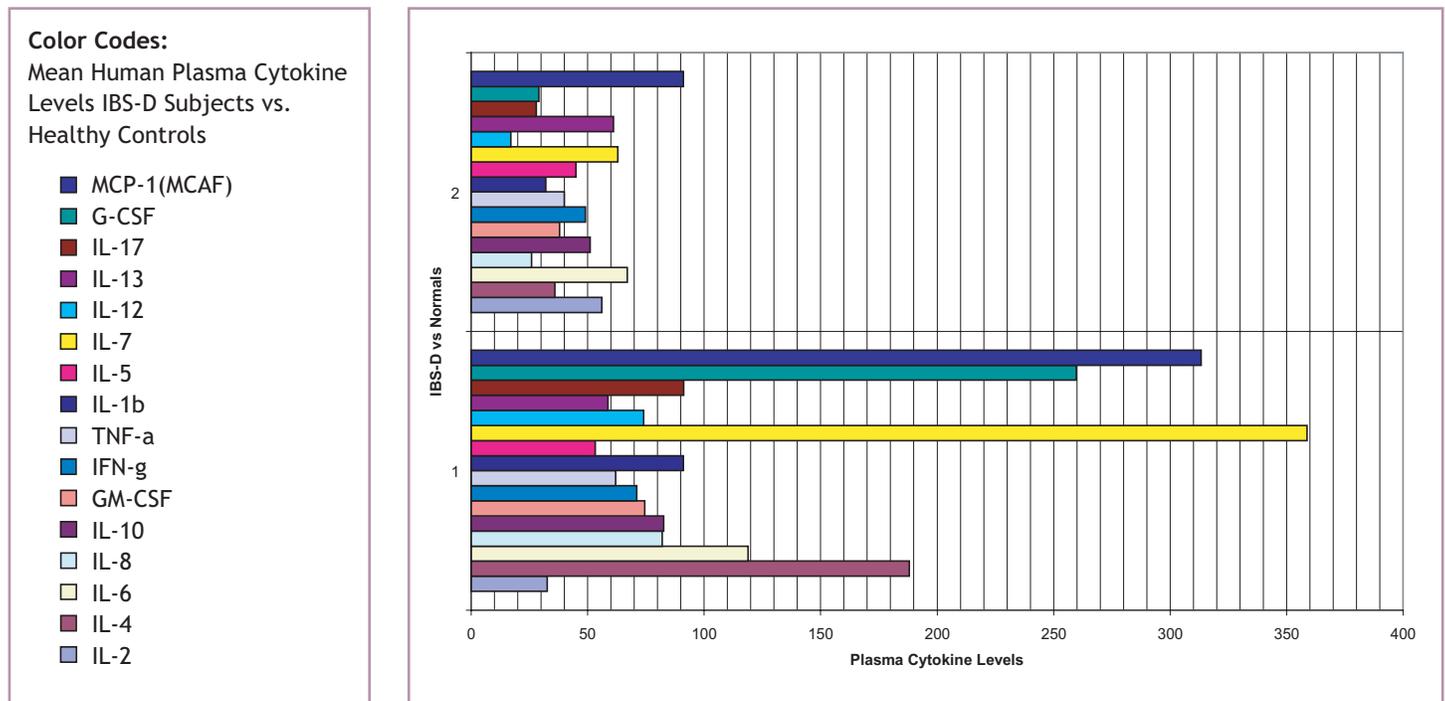


Figure 6

While there were various patient-specific differences, nevertheless a striking trend appeared. Comparing the baseline levels of these plasma cytokines in the asymptomatic controls to the cytokine levels in the IBS-D patients, the findings were consistent with those previously published (in vivo antigen challenge and analysis in non-atopic IBS-D subjects absent any circulating IgE antibodies to the provoking foods).

These discoveries, and the clinical experience with dietary therapy based on Mediator Release Testing, strongly suggest proinflammatory cytokines are implicated as intracellular mediators released in response to antigen challenge by symptomatic IBS-D subjects; that their release can be detected economically by the common end point MRT assay; and that these mediators are implicated in the symptomology of D-IBS. A number of specific cytokines were consistently elevated, though no specific pattern (other than proinflammatory) emerged which would suggest a single cytokine marker, rather an array of dysfunction can be observed.

Signet is preparing to engage in controlled clinical trials of the MRT-based LEAP Program to determine clinical efficacy as treatment for D-IBS. Further investigation into the role of proinflammatory cytokines and their inappropriate release as a consequence of lost oral tolerance to benign antigens in the D-IBS patient is clearly warranted, and will be included in the study design.

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