Study Title: Effect of Low FODMAP Diet on Colonic Epithelial Physiology in Diarrhea-predominant Irritable Bowel Syndrome

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PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	Effects of low FODMAP diet on leaky gut and mucosal immune cell abundance in diarrhea-predominant irritable bowel syndrome
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1. PURPOSE OF PROTOCOL

The pathophysiology of Irritable bowel syndrome (IBS) is multifactorial involving complex interplay of altered intestinal permeability, mucosal immune activation, visceral hypersensitivity and gut dysbiosis. Although the exact triggers for these pathological changes in IBS are not clear but diet might play an important role. In-fact, the majority of patients with IBS report dietary factors as triggers for gastrointestinal symptoms. Fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) have been shown to induce IBS symptoms. Several studies have reported improvement in gastrointestinal symptoms on a diet low in FODMAPs (LFD) in patients with IBS, specifically in diarrhea predominant IBS (IBS-D). However, the mechanism of action of LFD is not well understood. Recent data from rodent studies have suggested an increase in colonic permeability and visceral hypersensitivity in response to high FODMAP diet which are reversed on a LFD. However, no human study has investigated the effect of LFD on colonic intestinal permeability. Although a few human studies have investigated the effect of LFD on serum cytokines and fecal microbiome, no study to date has systematically looked into the effect of LFD on mucosal immune cell abundance or mucosal microbiome. We hypothesize that LFD alters mucosal microbiome which, in turn, causes improvement in mucosal immune activation as well as intestinal permeability. In this study, we aim to evaluate the following effects of LFD in patients with IBS-D- i) colonic permeability ii) abundance of colonic mucosal T-cells and mast cell abundance iii) colonic mucosal and fecal microbiome.

2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Irritable bowel syndrome (IBS) is a gastrointestinal disorder characterized by abdominal pain and altered bowel habits which affects up to 11% of world population.[1] There is overwhelming evidence that pathophysiology of IBS is multifaceted involving intestinal permeability, mucosal inflammation, mast cell activation, and visceral hypersensitivity.[2,3] The exact triggers for these physiological changes in IBS are not clear but likely factors include diet and microbial dysbiosis.[2,3]

Dietary factors are known triggers of IBS related symptoms up to 65% of IBS patients report symptoms attributed to food.[4] In double-blind, randomized, placebo-controlled trials; fructose and fructans have been shown to induce IBS symptoms in a dose-dependent manner.[5] In fact, a diet low in fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) is the most extensively investigated dietary intervention in IBS.[6–8] A recent meta-analysis of seven randomized controlled trials comparing a low FODMAP diet (LFD) with various control interventions in 397 participants suggested a 31% greater likelihood in improvement of global IBS symptoms.[9] In patients with diarrhea-predominant IBS (IBS-D), Eswaran et al showed that those randomized to LFD had greater reductions in daily scores of abdominal pain, bloating, stool frequency, stool consistency, and urgency than the modified National Institute for Health and Care Excellence (NICE) diet.[10] Others have also shown objective improvement in stool consistency of IBS-D patients with LFD using King's stool chart.[6]

Despite the clinical efficacy of LFD in improving symptoms of IBS-D, its mechanism of action is not clear. Recently, Zhou et al have shown FODMAPs induce colonic tight junction dysfunction and visceral hypersensitivity in rat models, both of which are reversible when rats were fed an LFD.[11] They further showed that this effect of FODMAPs is mediated by microbial dysbiosis and elevated fecal lipopolysaccharide level.[11] However, studies evaluating the effect of LFD on colonic permeability of humans are lacking. Halmos et al have shown that LFD significantly decreases butyrate producing

bacteria and fecal butyrate levels in humans, a short chain fatty acid known to improve tight junction assembly.[12] Other studies have evaluated the effect of LFD on fecal microbiome. No studies have evaluated the effect of LFD on the luminal microbiome in IBS.[13,14] Studies have shown significant differences in intra-individual luminal and mucosal microbiome of patients with functional gastrointestinal disorders.[15] Thus, the exact effect of FODMAP on intestinal permeability and mucosal microbiome in humans is not clear and needs further evaluation.

Several studies have also shown an increase in the number of mucosal T-cells and mast cells in the colonic mucosa of IBS patients.[16] Mucosal inflammation has been associated with altered gastrointestinal motor function, and visceral hypersensitivity.[3] These sensorimotor abnormalities can occur even when inflammation is minimal and restricted to the mucosa.[3] Although diet is perceived as an important trigger for mucosal mast cell increase and activation reported in IBS-D, it has been poorly investigated. The above reported study by Zhou et al also reported increase in colonic mast cell and mononuclear cell abundance in rats fed on high FODMAP diet.[11] However, their study was limited to the colon and lacked detailed phenotyping of immune cells.[11] The effect of LFD on human colonic mucosal immune cell abundance has not been reported.

Given the gaps in knowledge, we aim to evaluate the following in patients with IBS-D

1. The effect of LFD on colonic permeability
2. The effect of LFD on abundance of colonic mucosal immune cells specifically T-cell and mast cell abundance
3. The effect of LFD on colonic mucosal and fecal microbiome

3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

Study Design

Forty consenting IBS-D patients and twenty consenting IBS-C patients will undergo a 7-day screening period beginning with the baseline visit. Patients fulfilling the inclusion and exclusion criteria will undergo a i) flexible sigmoidoscopy to obtain colonic mucosal samples from the recto-sigmoid junction for assessment of intestinal permeability, mucosal immune cell abundance, and mucosal microbiome ii) stool collection for microbiome analysis iii) serological markers of intestinal permeability and iv) saccharide excretion assay. They will then go on a 4-week low FODMAP diet followed by a repeat flexible sigmoidoscopy with biopsies, stool collection, serological markers of intestinal permeability and saccharide excretion assay.

Baseline Visit

- Informed consent will be obtained from the subjects
- Medical history including IBS history will be obtained from the subjects
- Blood samples will be obtained
- Subjects will be instructed on the online questionnaires (described below) to be completed for the duration of the study.
- Subjects will be given a stool collection kit for collection prior to their next visit. The stool will be collected from the subjects' home and brought with frozen icepacks to their next visit.
- Subjects will be given the lactulose-mannitol standard solution and be instructed on urine collection for the test. Subjects will drink the solution first thing in the morning on a day of his or her choice prior to the Initiation Visit and collect of all their urine in a jug for the next 24 hours.

Initiation Visit (7 days post-baseline visit +/- 7 day)

- Subjects will undergo a flexible sigmoidoscopy in MCRU. The flexible sigmoidoscopy will only be performed up to the rectum. Subjects are not required to prep for the procedure (e.g. no diet restrictions or laxatives). Sedation will not be given to the subjects.

- Twelve biopsies will be obtained from the recto-sigmoid junction.
- Subjects will be given the low FODMAP diet instructions. Subjects will be instructed to stay on the low FODMAP diet for 4 weeks.
- Subjects will be given the lactulose-mannitol standard solution and be instructed on urine collection for the test. Subjects will drink the solution first thing in the morning on a day of his or her choice in the week prior to the Post-Diet Visit and collect of all their urine in a jug for the next 24 hours.
- Subjects will be given a stool collection kit for collection prior to their next visit. The stool will be collected at the subject's home in the week prior to the Post-Diet Visit and brought with frozen icepacks to the Post-Diet Visit.

Post-Diet Visit (28 days post-Initiation Visit +/- 5 days)

- Subjects will undergo a flexible sigmoidoscopy in the MCRU. The flexible sigmoidoscopy will only be performed up to the rectum. Subjects are not required to prep for the procedure (e.g. no diet restrictions or laxatives). Sedation will not be given to the subjects.
- Twelve biopsies will be obtained from the recto-sigmoid junction.
- Subjects will return the urine collection container.
- Blood samples will be obtained

Low FODMAP diet

Subjects who consent to participate in the study will be given low FODMAP meals delivered to their home by Epicured at no charge to the subject. Epicured is a meal delivery service that provides ready-to-eat low FODMAP meals. Subjects will be required to provide their home address and name to Epicured in order to receive the delivery 2-3x/week. Epicured will provide 3 meals, 2 snacks and 1 non-water beverage to subjects for each day of the 4 week low FODMAP period. Vegetarian, vegan, and omnivore meals will be provided. Subjects will be told to only ingest Epicured provided items (excluding water).

Healthy Control Arm

The stool of healthy subjects will be collected to compare to the IBS subjects' stool (details of analysis below). These subjects will be recruited from UMHealthResearch.org. Their medical records will be used to ascertain eligibility and to collect demographic information. The study team will consent and give the subject a stool collection kit. The subjects will return the stool to the study team and their participation in the study will end at that time.

IBS-C Arm

Like IBS-D, the physiological effects of a low FODMAP diet are unclear in patients with IBS-C. The relationship between the physiology of IBS-C and IBS-D, if there any, is also unclear. Thus, as a disease comparison group, 20 patients with IBS-C will be enrolled in the study in order to better understand how a low FODMAP diet effects and changes the microbiome and intestinal permeability of IBS-C patients.

In vivo Intestinal Permeability:

Saccharide excretion assay (Lactulose-Mannitol assay): The test is based on timed urine collection after ingestion of an oral load of two saccharide probes, lactulose and mannitol. In brief, subjects will be required to refrain from ingestion of alcohol, aspirin and NSAIDs for at least 7 days prior to the test. They will also be asked not to consume artificial sweeteners, lactulose, mannitol (2 days prior to the testing) and during the 24-hour testing period. A standard oligosaccharide solution of lactulose (1000 mg), mannitol (100 mg) and flavored rink crystals (1.5 gram) in 250 mL of bottled or tap water, followed by 500 cc of bottled or tap water after 30 minutes, will be ingested. Urine collections will be performed in containers with 5 mL of thymol solution for 0-2, 2-8 and 8-24h following the administration of test sugars and returned to the study center. The total urine volume will be measured, and urinary concentrations of lactulose and mannitol will be measured using high pressure liquid chromatography. The fractional excretion of lactulose and mannitol will be calculated

as the ratio of the total urinary excretion of the respective saccharide probe to the total oral dose of the probe. For each subject, the lactulose–mannitol ratio (LacMan ratio) will be calculated as the fractional excretion of lactulose divided by that of mannitol.

mRNA quantitation

We will use real-time PCR to quantitate mRNA expression of TJ proteins [ZO-1, occludin, claudin-1, 3, 8, 15] and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, control) in colonic biopsies from IBS-D patients.

Colonic biopsies will be submerged in RNAlater Solution (Ambion, Austin, TX) and stored at −80°C. RNA extraction will be performed as in the manufacturer's instructions (RNeasy Mini Kit, Qiagen, Valencia, CA). cDNA synthesis will be performed using 0.2 µg of total RNA with the High Capacity Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Taqman gene expression assays for ZO-1, occludin, claudin-1, 3, 8, 15 and GADPH will be carried out in duplicate for each gene on an ABI Prism 7900HT Real-time PCR System (Applied Biosystems) according to manufacturer's instructions using the comparative $^{\Delta\Delta}$CT method for relative quantification. The expression of each gene will be normalized to the endogenous control, GAPDH. This will be performed at the Owyang Lab at U of M.

Immunofluorescence for tight junction proteins contract lab

Colonic biopsies obtained pre- and post-dietary intervention will be used for this study. Formalin-fixed tissues will be embedded in paraffin and assembled into tissue microarrays before staining. After de-paraffinization, 5 µm sections will be rehydrated, and antigens will be unmasked by boiling in 0.01 M Tris/EDTA (pH 9.0). Slides will be stained with mouse anti-human ZO-1 monoclonal antibodies (catalog# 33-9100, Thermo Fisher Scientific), occludin polyclonal antibody (catalog# 71-1500, Thermo Fisher Scientific), rabbit polyclonal anti-claudin-1 (catalog# 51-9000, Thermo Fisher Scientific), rabbit polyclonal anti-claudin-3 (NBP1-48526, Novus Biologicals, Littleton, CO), rabbit polyclonal anti-claudin-8 (NBP1-59157, Novus Biologicals, Littleton, CO), rabbit polyclonal anti-claudin-15 (NBP2-13842, Novus Biologicals, Littleton, CO) primary antibodies. Primary antibodies will be detected using Alexa Fluor 594 or 488 conjugated secondary antibodies or highly purified secondary Fab'$_2$ fragments (Jackson Immunoresearch). Nuclei will be labeled with Hoechst 33342 (H3570, Thermo Fisher Scientific). Stained sections will be mounted in Prolong Diamond (Thermofisher Scientific). Tiled images will be collected on a DM4000 microscope equipped with Chroma filter sets (as above), a 20× HC PLAN APO NA 0.7 objective, CoolSNAP HQ2 grayscale CCD (for fluorescence) and Jenoptik ProgRes C10 Plus color CCD (for transmitted light, i.e., hematoxylin and eosin-stained sections) cameras, and a motorized *xyz*-stage (Ludl) controlled with custom MetaMorph 7 journals. Post-acquisition processing, including stitching of tiled images and quantitative analysis of signal intensity, will be performed using Metamorph 7 (Molecular Devices Sunnyvale, CA). The samples for this testing will be sent out coded without patient identifiers to tge Owyang Lab at U of M.

Serological tests for intestinal permeability

Following serological markers for intestinal permeability will be assessed: serum lipopolysaccharide (LPS) activity measured with the Limulus Amebocyte Lysate assay (LAL, Hycult Biotechnology, the Netherlands), and serum zonulin levels (Catalog number 30-ZONSHU-E01; ALPCO, Salem, NH). This will be performed at the Owyang Lab at U of M.

Intra-epithelial and lamina propria mononuclear cell isolation

Mononuclear cells will be obtained from colonic intra-epithelial and lamina propria fractions. Intra-epithelial (IELs) and lamina propria lymphocytes (LPs) will be isolated following incubation in dissociation buffer (HBSS Ca^{2+} and Mg^{2+} free containing 2% FBS, 5mM EDTA, 10 mM HEPES) and digestion buffer (HBSS with Ca^{2+} and Mg^{2+}, 10% FBS, 0.5 mg/ml DNase I and 0.5 mg/ml collagenase type IV), resuspension in 40% and layering on 80% Percoll Plus. IELs and LPs viability will be checked by Trypan blue exclusion.

Flow cytometry staining

Cell phenotype will be assessed by flow cytometry following cell incubation with fluorochrome-conjugated anti-human antibodies to: CD3, CD4, CD8, CD45RO, CD45RA, CD25, CD39, CD73, CD56 and CD117 (or c-KIT, marker of mast cell). Frequency of cells positive for FOXP3, RORC, T-bet and GATA-3 - transcription factors of Tregs, Th17, Th1 and Th2 lymphocytes will be assessed by intracellular staining following cell fixation and permeabilization with Cytofix/Cytoperm and incubation with fluorochrome-conjugated anti-human FOXP3, RORC, T-bet and GATA-3. Frequency of cytokine-producing cells will be determined after exposure to phorbol 12-myristate 13-acetate (PMA, 10ng/ml) and Ionomycin (500ng/ml) for 60 minutes and to Brefeldin A (20µg/ml) for additional 5 hours. Cells will be acquired on a BD LSRII and analyzed using BD FACSDiva software. $3\text{-}5\times10^4$ events will be acquired for each sample. Positively stained cell populations will be gated based on unstained, single stained and isotype stained controls. This will be performed at the Owyang Lab at U of M.

Immunohistochemistry for immune cells

For immunohistochemistry, 5µm colon biopsy sections will be stained for human CD3, CD4, CD8, CD56, CD39, FOXP3, RORC, T-bet, GATA-3 and CD117 (or c-KIT). Endogenous peroxidase blocking with 3% H_2O_2 will be carried out. Sections will be incubated overnight at 4°C with primary antibodies to the above antigens and subsequently exposed to avidin/biotin complex with HRP (Vector Laboratories, Burlingame, CA). ImmPACT DAB (Vector Laboratories) was then applied and sections examined by light microscopy. This will be performed at the Owyang Lab at U of M.

Mucosal and fecal microbiome

Two colonic mucosal biopsies and stool samples obtained before and after the dietary intervention would be used for this analysis. Once obtained, each biopsy will be washed using Sterile PBS to remove non-adherent luminal/fecal material. The biopsy samples will be weighed, flash frozen in liquid nitrogen and stored at -80C for DNA extraction and microbiological analysis. DNA extraction will be done using a QIAamp DNA Mini Kit (Qiagen, Crawley, UK) per manufacturer's instructions. Bacterial community composition in isolate DNA samples will be characterized by amplification of the V1-V3 variable regions of the 16sRNA gene by polymerase chain reaction for both mucosal and fecal samples. 16S rRNA PCR products will be quantified, pooled and purified for the sequencing reaction. 454 GS FLX Titanium sequencing will be performed on a 454 Life Science Genome Sequencer FLX machine for both mucosal and fecal samples. Sequencing data generated will be processed by the Quantitative Insights into Microbial Ecology Pipeline (QIIME). Sequences will be screened for chimeras using the ChimeraSlayer algorithm, and all potential chimeras will be excluded from downstream analysis. Sequences less than 200 bp or greater than 1000 bp in length, those with average quality scores <20, incorrect primer sequences, or more than 1 ambiguous base will be excluded from downstream analysis. The quality filtered sequences will be assigned to operational taxonomic units (OTUs; sequences that share ≥97% similarity) using a closed reference approach in QIIME with the UCLUST algorithm and the Greengenes reference database. According to the results of OTUs analysis, α diversity analysis including Simpson, Shannon, Chao 1, ACE and Good's Coverage will be carried out by QIIME. β diversity metrics, including (UniFrac weighed or unweighed) will also be calculated.

Stool samples will also be obtained from 30 healthy controls aged 18-65 years without any known gastrointestinal history and who do not report any abnormal bowel movements or abdominal pain. Stool samples from healthy controls will be utilized for analyzing fecal microbiome using the methodology described above. Their microbiome will be compared with microbiome of IBS-D patients pre and post LFD.

Clinical variables

Data will be collected and stored via Research Electronic Data Capture (REDCap), a HIPAA compliant, free, secure, web-based application. Patients will be automatically emailed a link daily (via REDCap) with a REDCap link to the questionnaires listed below.

Patients will be asked to complete the IBS-SSS and record their most common stool consistency of

the day (measured using Bristol stool form scale) every day during the baseline period and the last week of 4-week LFD. In addition, following questionnaires will be administered at the end of the baseline period as well as the end of the 4-week LFD.

Patient Reported Outcomes Measurement Information System (PROMIS)
The Patient Reported Outcomes Measurement Information System (PROMIS) is a National Institutes of Health (NIH) set of tools used to provide information on patient outcomes in a variety of fields.

Gastrointestinal PROMIS scales: PROMIS scales of Belly pain and diarrhea will be administered to assess the severity of belly pain and diarrhea in our patients. PROMIS Belly pain questionnaire and PROMIS diarrhea questionnaire have five and six questions, respectively, which assess symptom severity on a 5 point Likert scale. Higher T-scores on these questionnaires refer to more severe gastrointestinal symptoms.
PROMIS belly pain asks how often did you have belly pain, severity of belly pain, interference with activities, bothersomeness and discomfort.
PROMIS diarrhea asks how many days did you have loose stools, interference with activities, bothersomeness, and how often you experience urgency.

Irritable Bowel Syndrome Severity Scoring System (IBS-SSS): (IBS-SSS) is a validated scale for assessing overall IBS symptom severity.[22] It includes 5 questions of equal weight concerning symptoms over the past 10 days: average severity of abdominal pain, number of days with abdominal pain, average severity of abdominal distension or bloating, satisfaction with bowel habits, and the overall interference in their quality of life from these symptoms. All questions are scored on a 0-100 scale. The scores for all five questions are summed to a total IBS-SSS score between 0-500. Lower scores indicate lower symptom severity.

Diet compliance: Patients will also be asked to record their day's food and drink intake and record whether they were compliant with the FODMAP diet. Prior to the baseline flexible sigmoidoscopy, patients will be asked to record the previous 3 days' food and drink intake

B. Statistical Considerations

Sample Size Justification: **Statistical analysis**
All continuous variables will be tested for normality using the Shapiro-Wilk test. Normally distributed continuous data will be presented as mean (± SD) and will be compared pre-and post-dietary interventions using paired Student's t-test. Continuous data which are not normally distributed will be presented as median (range) and compared using Wilcoxon signed-rank test. Proportions will be expressed as percentages and compared using chi-square test or fisher exact test as appropriate. Since we anticipate that most of our tests will involve continuous data that is normally distributed, we based our power analysis on pre-post comparisons using paired t-tests. Assuming two-tailed tests, with alpha set at 5%, a sample size of 20 will provide 80% power to detect a medium effect size (i.e., Cohen's d=.5) when the pre-post correlation is r=.7.

C. Subject Selection

Inclusion criteria for IBS subjects

Patients with IBS-D or IBS-C diagnosed per Rome IV questionnaire and without any alarm features (rectal bleeding, weight loss, nocturnal symptoms, family history of inflammatory bowel disease or celiac disease) will be considered for the study if they had:

i) Aged 18-65 years at the time of screening
ii) Normal serum studies including serum tissue-transglutaminase antibodies, thyroid stimulating hormone levels, C-reactive protein, complete blood count since the onset of symptoms
iii) Normal stool studies including, ova and parasites since the onset of symptoms
iv) IBS-SSS score of ≥175 at the end of the 7-day screening period

In case of presence of any alarm features and/or elevated inflammatory markers (C-reactive protein or fecal calprotectin), patients will be eligible if they have been excluded for inflammatory bowel disease with upper endoscopy, and colonoscopy in the last one year.

Exclusion criteria for IBS subjects

i) individuals already on a LFD or other dietary restriction such as gluten free diet within the past 6 months
ii) individuals with any known food allergy or insulin-dependent diabetes
iii) known history of celiac disease, inflammatory bowel disease or microscopic colitis
iv) prior small bowel or colonic surgery or cholecystectomy
v) pregnant patients
vi) Antibiotics in the past 3 months
vii) Those who regularly use mast cell stabilizers or anti-histaminic or non-steroidal anti inflammatory agents (NSAIDs) excluding daily baby aspirin or steroids or bile-acid binder.

Inclusion criteria for healthy volunteers

Aged 18-65 years

Exclusion criteria for healthy volunteers

1. History of gastrointestinal illness
2. Self-reported constipation, diarrhea, or abdominal pain
3. Antibiotics in the past 3 months
4. Gastroenteritis in the past 3 months

4. POSSIBLE BENEFITS

No direct benefit to subjects is expected, although there may be some relief from IBS symptoms. However, this will help understand the pathophysiology of IBS-D and investigate the effect of low FODMAP diet on various physiologic parameters and even identify the predictors for response to this dietary intervention.

5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

The possible risks for the study include flexible endoscopy related complications such as bleeding or perforation. These are very unlikely to occur as we will be examining and taking biopsies from the last 20 cm of colon (and not performing polypectomies). These complications are very rare and unlikely to happen.

Blood Drawing
The risks and discomforts of blood drawing from a vein include the possibility of pain or bruising at the site of the blood draw; occasional feeling of lightheadedness; and rarely, infection at the site of the blood draw.

The benefit of this study is to help us understand how low FODMAP diet impacts the various gut functions- permeability, immune activation and microbiome. This will help us identify the patients who might benefit from this dietary intervention (as the diet is cumbersome to follow). Will also shed light on how diet interacts with gut physiology.

6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment
Patients will be identified in the following ways:

- Clinical practice at Michigan Medicine
- GI referrals
- Review of medical records and appointment logs
- Advertisements
- UMHealthResearch.org
- Data Direct screens
- EMERSE screens

Potential study participants (obtained from the means listed above) will be approached or contacted by phone or email. A brief screening will be initiated to assess subject's eligibility before scheduling the office visit. Patients identified through clinical screening will be called using a phone script. Patients identified through a data direct / EMERSE screen will be re-screened and called by study staff to judge their eligibility and interest. Patients will be contacted three times before study team will cease contact.

Consent
During the Baseline Visit, a member of the study team will fully explain the purpose of the study to the patient and all questions and concerns regarding the study will be addressed as well (informed consent process). This will take place in a private area.

Subject Protection
None of the subjects in this study will be vulnerable to coercion or undue influence

7. STUDY LOCATION and DATA SECURITY

Privacy
All data will be kept in a password protected folder on the shared drive. All serum, urine and biopsy samples will be coded without patient identifiers and stored in restricted-access research areas at U of M. When the mucosal samples will be sent to a contract lab, they will not have any PHI labeled.

Physical Setting
All samples will be stored in freezers in restricted-access areas. All data generated from this study will also be stored in a password protected folder or in locked cabinets in locked offices.

8 Dissemination of Research Results

Patients will not be informed about the results of the study. Results might be presented/published.