Immunology of Celiac Disease

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Abstract

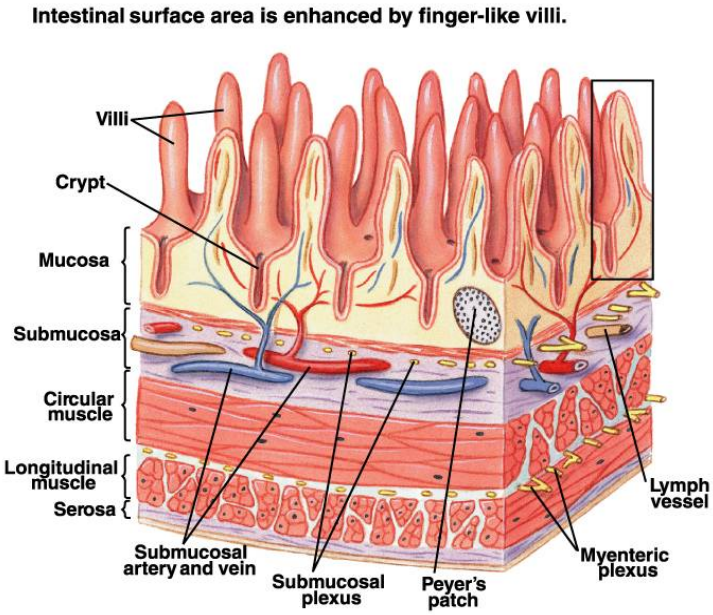
The basis of this experimentation is to detect the cytokines levels in four groups of patients; 1 control and 3 variable groups. Our control was healthy patients, those without Celiac Disease. The three variables were as follows; patients with Celiac Disease, patients with Celiac Disease, but on a Gluten-Free Diet, and those with Refractory Disease. These groups will be explained in more depth later.

The cytokine that were checked for are the following; Interferon-γ (IFN-γ), Interleukin-1 (IL-1), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p70, and Tumor Necrosis Factor-α (TNF-α). Most of theses are pro-inflammatory cytokines. Pro-inflammatory cytokines are mainly produced by active immune cells and promote redness, swelling, pain, and a feeling of heat that help protect tissues affected by injury, disease, or infection.

Research

Celiac Disease is an autoimmune intestinal disorder, meaning the body's immune system attacks its own intestines. This is also hereditary to an extent, as family member of those with Celiac Disease are found to have a higher chance of having the disease themselves than others. It is predicted to be associated with a group of genes on Chromosome 6. Currently, 1 out of 133 people in the United States have this disease, making it very common, but very few actually get diagnosed for it. This is due to a higher rate of misdiagnosis than in other disease. It is very similar to such diseases as irritable bowel disease and chronic digestive disease, as they have the digestive problem symptoms. It has also been misdiagnosed for Crohn's disease, chronic fatigue syndrome, and Diverticulosis.

Due to this high rate of misdiagnosis, people are trying to find more accurate ways of diagnosing this disease. One such way was measuring the cytokine levels in people with this disease and looking for any correlation between pro-inflammatory cytokine levels and Celiac Disease.

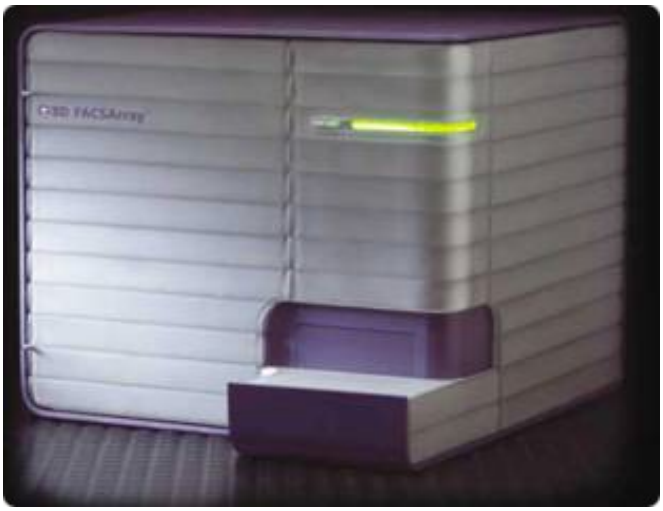


The only treatment for people with Celiac Disease is a very strict, gluten-free diet. Gluten is found in wheat, rye, and barley. Patients are given a very strict list of what they

may and may not eat. This is because gluten is the activator of this disease as it has a protein called glaidin. This protein attaches tot the HLA-DQ2/DQ8 receptor of an antigen-presenting cell. In turn, this attracts a helper T cell which attaches to the glaidin attached to the antigen presenting cell. The helper T cell promotes the production of antibodies and cytokines, in the plasma cells and the lymphocytes respectively. These antibodies and cytokines, instead of attacking the glaidin, attack the enterocytes of the gut lumen, removing the only layer of protection for villi in the gut lumen. The villi then gradually disappear. Without the villi adding absorption surface area, malnutrition is imminent.

Since a gluten-free diet is found to end all physical symptoms of the disease and allows the villi to rebuild themselves, the patients on this diet will most likely have the same cytokine levels as the control group, after a week or two of being on the diet.

Refractory Disease patients are those that are on the gluten-free dies, yet it doesn't seem to be helping them. It is unknown why this occurs in some patients and not others, but it is hypothesized that these patients will have the same cytokine levels as active celiac disease patients.



The equipment being used includes a flow cytometer. This device's components include a flow cell, where the serum that was used was put in to be analyzed, and a light source, which is shined at the liquid stream. The device then

detects light particles being reflected back to the light source. Some of it is scattered light, while there is still fluorescent light also. The reflected light is reflected off capture beads, which comes in many varieties depending on what is being detected in the liquid stream. Capture beads which attach to the cytokines that were being testing, and reflect light at a different wavelength depending on what cytokine is attached to it, were used. This technology can also be used for cell counting, DNA, RNA, proteins, antigens, as well as many other particles we would not be able to detect otherwise. The cytometer that was employed used a 96-well plate, making it much easier for us, as we had many samples to analyze. The results are saved on a spreadsheet on the computer and are then analyzed using Excel.

Introduction

In this experiment, following cytokine levels will be tests in the following groups of patients; Health Controls, Active Celiac Disease patients, patients on a gluten-free diet, and patients with refractory disease. The cytokines that were tested are IFN-γ, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12 p70, TNF-α.

Methods

- Dilution of Standards

Standards were used to calibrate the flow cytometer. The Standards were diluted by 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:125, 1:250, and 1:500. The standards were just FACS buffer solutions in water.

- Calibration of Cytometer

To do this, we had solutions of just the cytokines we were testing. After this we calibrated the equipment so it knows which wavelengths of light represent which cytokines.

- Excel Spreadsheet

To analyze the results, and make them less of an eyesore, we used Excel spreadsheet to put the data into graph format.

Materials

- Flow Cytometer
- 96-Well Plate
- BD™ Cytometric Bead Assay (CBA) Human Inflammation Kit
- BD™ CBA Th1/Th2 Kit
- 54 Serum Samples from Patient Biopsies
- FACS Buffer Solution
- Computer w/ Flow Cytometer Software and Excel Software

Experimental Procedure

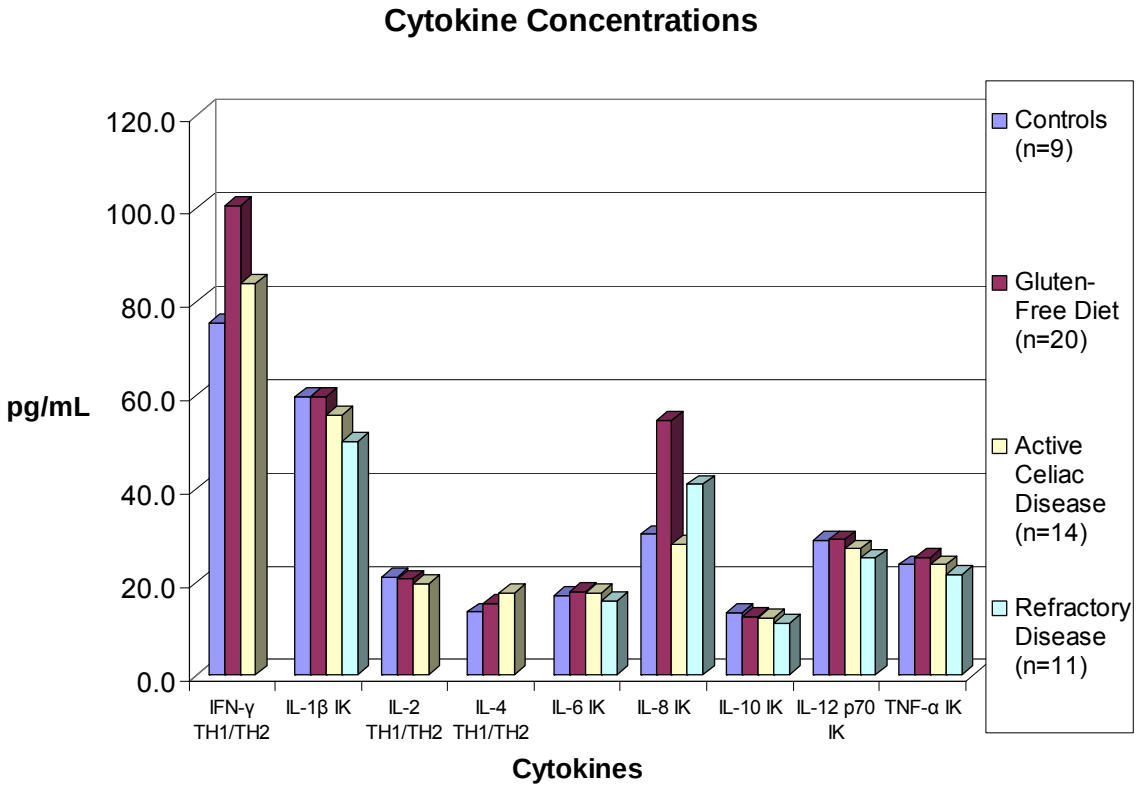
- Culture Supernatant Assay Procedure

1. Reconstitute Standards in Assay Diluent (15 min).
2. Dilute Standards by serial dilutions using the Assay Diluent.
3. Mix 10 μL/test of each Capture Bead suspension (vortex before aliquoting).
4. Transfer 50 μL of mixed beads to each assay tube.
5. Add Standard Dilutions and test samples to appropriate sample tubes (50 μL/tube).
6. Add PE Detection Agent (50 μL/test).
7. After a 3 hour incubation period at room temperature in the absence of light, wash samples with 1 mL Wash Buffer and Centrifuge.
8. Add 300 μL of Wash Buffer to each assay tube and analyze samples.

- Cytometer Setup Bead Procedure

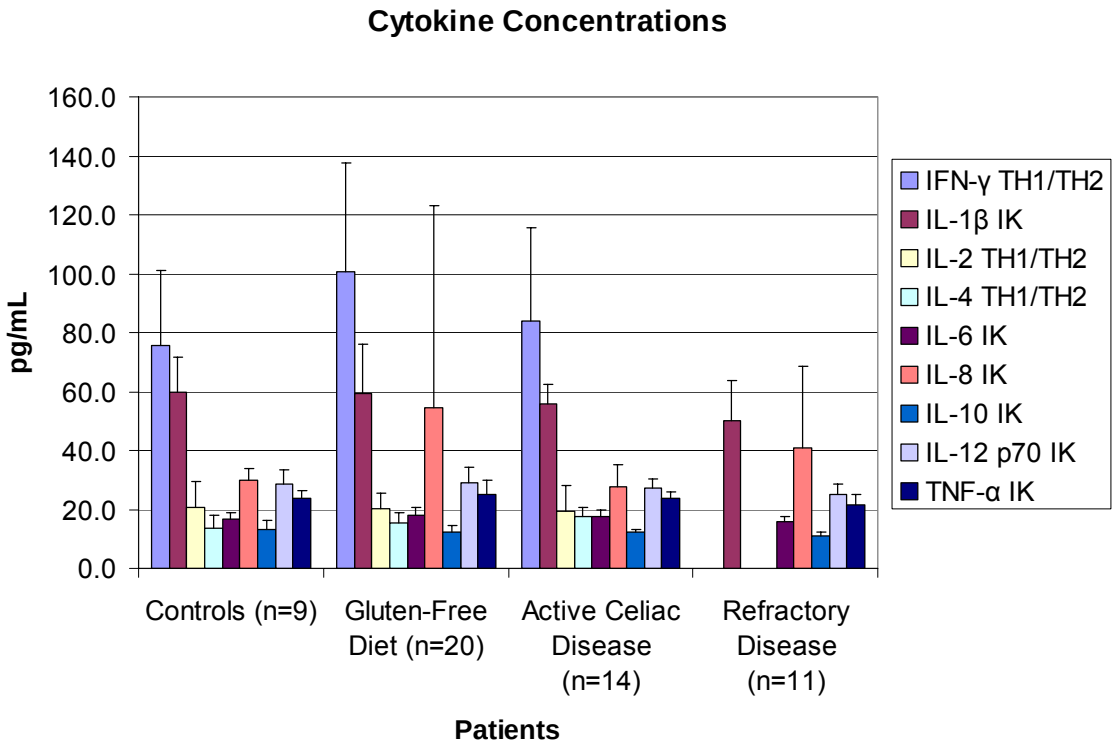
1. Add Cytometer Setup Beads (vortex before adding) to setup tubes A, B and C (50 μL/tube)
2. Add 50 μL of FITC Positive Control to tube B and 50 μL of PE Positive Control to tube C.
3. After a 30 minute incubation period at room temperature in the absence of light, add 400 μL of Wash Buffer to tubes B and C.
4. Add 450 μL of Wash Buffer to tube A.
5. Use tubes A, B and C for cytometer setup.

Results



*Note – Where there are no bars to denote values (e.g.: IFN-γ Refractory Disease) the results for this test was marked void do to lack of fluid.

-Standard Deviations Chart



*Note – Where there are no bars to denote values (e.g.: Refractory Disease IFN-γ) the results for this test was marked void do to lack of fluid.

Discussion

All the cytokine levels seem to be level through out except that of IFN-γ, where there are increased levels in Active Celiac Disease patients, and those on a gluten-free diet, and in IL-8, where there is a much larger concentration in patients with gluten-free diets. This was unexpected as one would think that since there are no physical symptoms of the disease, there wouldn't be any internal differences between healthy patients and those on a diet.

This was perplexing, so we decided to look at the data charts and found out that there were only a few gluten-free diet patients with very high cytokine levels, while the others were at a normal level. A standard deviation chart was made to show this. As one can see, the standard deviation in patients on a gluten-free diet varied greatly.

It seemed as the gluten-free diet was not working to well for some patients, which were the extremes that raised the average, but the rest had a normal level of cytokines. These patients will be looked on by patient-to patient basis to see what could have caused these large cytokine values, or if the diet is actually helping, as they might have acute refractory disease.

Conclusion

Pro-inflammatory cytokines such as IFN-γ and IL-8 seemed to be increased in both patients with active disease and Celiac Disease patients on a gluten-free diet. This is due to the fact that inflammatory reactions are on the rise as they try to rebuild the destroyed villi.

Now to get a more accurate view of the results, we will look at them on an individual patient-by-patient basis.

Future Work

We must now analyze the results on a patient-by-patient basis, and take into account any other factors that might affect cytokine levels in the sera. With those results, we will be able to find average cytokine levels in people with and without Celiac Disease, making it easier to diagnose for this disease, with a smaller chance of misdiagnosis.

Acknowledgements

- Dr. Sanil Manavalan - Mentor
- Dr. Jamal Naiyer – Co-mentor
- Sindhu Kunduvaram – Undergraduate Student
- Dr. Sat Bhattacharya – Coordinator OF HCS
- Mrs. Susan Farenholtz – Coordinator of Project Seed
- Harlem Children Society
- Project Seed

References/Further Reading

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