Is it possible to cure the inflammatory immune response and need for a gluten-free diet in Celiac Disease sufferers?

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Introduction

Autoimmune diseases, are diseases in which the immune system attacks itself leading to serious symptoms, sometimes organ failure, and even death[8]. The largest problems associated with autoimmune diseases are the weakening of the entire body and the lack of cures and treatments to the many forms of these diseases. The focus of this proposed research will be to cure one of the most common of autoimmune diseases, known as Celiac Disease, through temporary parasite infection. These parasites, better known as hookworms, are common in contaminated soil and water in countries that lack a good water treatment or sewage system[2]. Hookworms have been studied for their secretory products, which are believed to lessen allergies and inflammation related inflictions[14]. Some research has been conducted to study the ability of these secretory products that help allergy sufferers to help patients with Celiac Disease. This research proposal will focus on the prospect of using *Ancylostoma duodenale* hookworm to treat the inflammatory immune response caused by ingestion of the gluten protein in CD patients.

Celiac Disease (CD)

CD is an inflammatory autoimmune disease that, if left untreated, can have detrimental effects on the duodenum of the small intestine. The immune response in CD is controlled by CD4 T cells specific for peptides that become deamidated by transglutaminase 2 (TG2) enzyme, the target of the autoantibodies in CD[11]. This disease is linked to increased mortality and morbidity in untreated patients[5]. A minority of CD patients present asymptomatic[5]. The only successful treatment of this disease, at this point, is a diet banning the consumption of food containing the gluten protein found in wheat, barley, rye, and some other grains[10]. Three days on a diet with gluten results in the emergence of the symptoms in the duodenum[10]. The diet puts the disease into remission until the gluten protein is again ingested. CD is the most common autoimmune disease that can be caused by either environmental factors weakening the immune system and or the genetic factor human leukocyte antigen (HLA) allele[5].

The diagnosis of CD often follows several misdiagnoses due to the outward symptoms' commonality[5]. Symptoms associated with CD vary from malabsorption leading to malnutrition, diarrhea, weight loss, osteoporosis, iron and folic acid deficiency, athralgia, fatigue, brain fog, and abdominal pain[11]. The less invasive way of diagnosing CD is serological testing of the blood for proteins specific to CD and pro-inflammatory cytokines such as immunoglobulin A (IgA), immunoglobulin G (IgG), CD specific autoantigen, and TG2. The pro-inflammatory IgA and IgG and the CD antibodies are not in excess in the blood work of patients on a gluten free diet[11].

A duodenal biopsy is conducted for four pieces of tissue to ensure that the patient has CD[5]. The small intestinal morphology of a CD sufferer tends to have lesions from the malabsorption and autoimmune response marked by intraepithelial lymphocytosis[5]. The biopsies of the duodenum show whether or not the mucous lining and villi is small or missing as

compared to healthy duodenum samples[13]. In CD patients, the wall of the duodenum lacks mucous and long villi leading to the malabsorption[11]. The biopsy conducted to diagnose CD is an esophagogastroduodenoscopy which involves a lighted probe needle down the throat through the esophagus and the stomach to the duodenum where the tissue is cut out[13]. Fasting and laxative purging to clear out the gut is needed to be conducted one day before the procedure to ensure clean pictures[13].

The cases of CD discovered and estimated worldwide were well documented and laid out in the World Journal of Gastroenterology, Volume 18 Issue 42, "Celiac Disease: Prevalence, Diagnosis, Pathogenesis, and Treatment". The data from that paper has been compiled into table 1 below.

Table 1: Estimated CD Prevalence Worldwide

Region	Prevalence in Percent
Africa	0.3-5.5
Asia	0.3-1.0
Australia	0.4-1.2
Europe	0.4-1.1
Middle East	0.6-1.1
North America	0.5-1.0
South America	0.1-1.5

This table shows the lower and higher estimated number on people suffering from CD worldwide based on extensive studies. The region with the most estimated people inflicted with CD is Africa followed by South America, Australia, Europe, Middle East, Asia, and North America [5]. This datum implies that somewhere between 2.6 and 12.4 percent of the world's more than 7 billion people (168-868 million people) suffer from CD. The upper bound of the estimated percent of people estimates almost 1 billion people having CD[5].

***Ancylostoma duodenale* (*A. duodenale*)**

A. duodenale is a species of hookworm parasite from the helminth family of nematodes that is transmitted via the oral-fecal route and infects the gastrointestinal system of humans that have come into contact with contaminated water[6]. These infections are prevalent in impoverished and coastal areas and are most popularly treated with Albendazole to poison the parasite[6]. Albendazole is an anti-parasitic drug that acts to alter the intestinal tissue of the parasites through tubulin binding, inhibiting the ability to polymerize microtubules in the cytoskeleton, and leading to death[12]. The usual course of Albendazole for *A. duodenale* is 400 milligrams for two weeks[12]. Infections of these parasites causes elevated levels of immuglobulin E (IgE) in the blood and iron deficiency often occurs from intestinal bleeding[6]. IgE is an antibody of the immune system which is mostly associated with type 1 hypersensitive associated with allergies.

The excretory and secretory products of hookworms repress intestinal pathology inducing clusters of differentiation 4 (CD4) helper T cells, also known as leukocytes, to divide and secrete interleukin 4 (IL4) and Interleukin 10 (IL10) cytokines[3]. Cytokines are known to assist in active immune response[3]. The IL4 cytokine is a naive T helper cell that induces differentiation into active B-cells. B-cells are important for adaptive immune response; because they bind antigens enabling the ability of the immune system to create antibodies with plasma cells. IL10 is an anti-inflammatory cytokine, which binds to receptors blocking

the ability of the pro-inflammatory cytokines of the immune system[3]. Hookworm can live for years in the human intestines causing various problems from daily discomfort to chronic anemia and can often lead to death[4].

A. duodenale was chosen as the nematode parasite for this study, because it is the only known nematode to only target the duodenum of humans[6]. The symptoms of this hookworm are minimal, normally no worse than minor diarrhea, and worsens over time[6]. *Nectator americanus (N. americanus)* is the hookworm that has been used in previous studies with CD[1,2,4,9] but *A. duodenale* has been discussed as an alternative with possibilities for more consistent results[6].

CD with induced *A. duodenale*

Little research has been done to see if the ability of hookworms to induce the immune system response of humans can temporarily or permanently cure CD. The research that is circulating mainly deals with infection of *N. americanus* hookworm[1,2,4,9], which is not specific to the duodenum as is *A. duodenale*. *N. americanus* is also popular in the literature as being tested for the possibility of curing allergies[2,14]. This hookworm can live in the lungs, brain, duodenum of the small intestine, and in the blood[7]. Its symptoms are similar to *A. duodenale* and can cause pain, bleeding, and discomfort from diarrhea, but is more serious because it sometimes causes serious lung infections leading to death[1].

The study conducted by the McSorley lab in 2011 tested twenty patients with CD concluded that ten patients induced with hookworm did not have suppression of the pathological immune response, but did have suppression of the mucosal inflammatory response[9]. This study found elevated levels of IL10 in the patients, which is known to be an excretory product of hookworm that decreases inflammation[9]. Another study conducted by the McSorley lab in 2012 tested twenty patients with CD for an increase in the cytokine response upon hookworm infection and also found a decrease in the mucosal inflammatory response due to the cytokine IL10[4]. A study conducted by the Daveson lab in 2011 studied twenty patients in a double-blind study with half being infected with hookworm and this study concluded that both groups had severe deterioration of the duodenum after a wheat challenge test[2]. A wheat challenge test just being a diet containing increasing amounts of gluten over time[2].

A. duodenale causes an increase of many immune system cells in the human intestinal system. One of these types of cells is IgE, which is seriously deficient in CD sufferers[4]. Hookworms also release IL10 and IL4 into the blood stream of the host[3]. Together these two cytokines allow for the creation of antibodies and the ability to avoid inflammation[3]. Inflammation is one of the most common and intense symptoms of CD patients when they ingest gluten[3]. Two of the previous studies conducted found the elevated IL10, but did not report on IL4, HLA, TG2, or CD4.

Objectives

This research proposal aims at testing the hypothesis that if CD patients are infected with *A. duodenale* then the secretory products of the nematodes will cure the inflammatory immune response temporarily or permanently and the need for a gluten-free diet.

The hypothesis will be tested through conducting a double-blind study involving

infecting CD sufferers with *A. duodenale*. These patients will be tested for the secretory products of the *A. duodenale* and for the immune system response that is normal in CD patients when they consume gluten. Duodenum samples from the patients will be obtained and monitored for histological changes in order to be able to conclude if the nematode infection ends the inflammatory immune response.

Research Objectives

- Observe immune cells specific to *A. duodenale* and CD sufferers in blood work
- Observe changes and damage to the tissue of the duodenum
- Test possibility of *A. duodenale* secretory products to have long-lasting to permanent effects

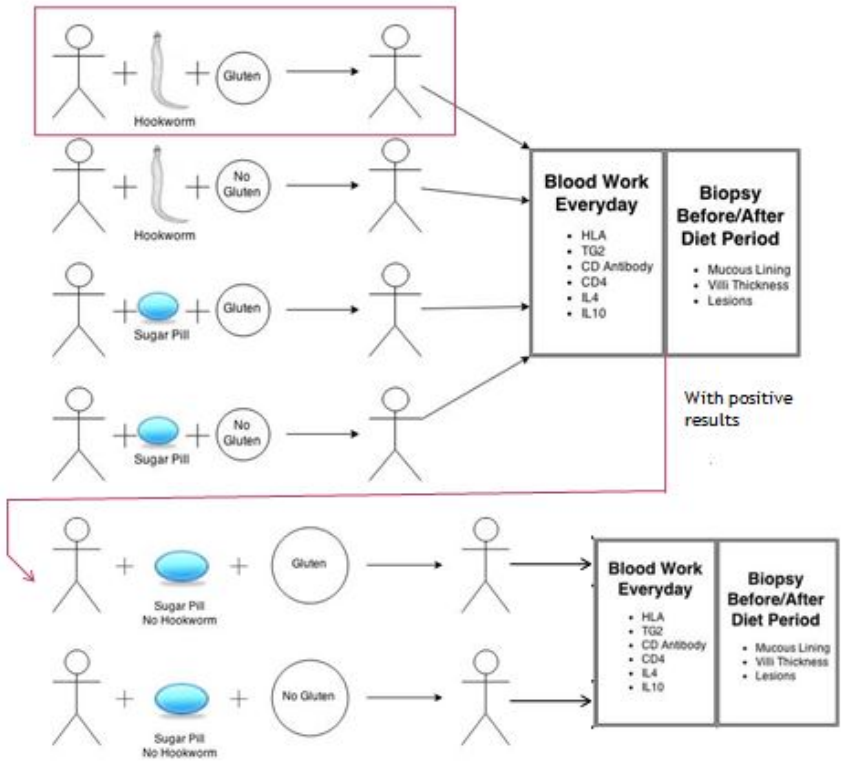

Figure 1: Research Scheme

Double-blind study with four groups: two groups with *A. duodenale*, two groups without *A. duodenale*, and one group from of each of those subgroups with gluten. All participants will have daily blood work and biopsies before and after each diet period. If the results for the group given *A. duodenale* and gluten are positive this group will be used in a second double-blind study. They will be split into two groups, neither receiving *A. duodenale*, one group will ingest gluten and one no gluten while being tested for changes in blood and duodenum.

Research Approach

This experiment will be conducted in hospitals around the world with collaborators, the majority of hospitals participating will be in countries with more instances of CD (seen in Table 1). Sample sizes between one-hundred and one-thousand will be used, depending on locality. The participants will be monitored on a gluten-free diet for three months with biopsies before and after the preliminary controlled diet is started, to ensure their disease is being well controlled with the diet and for a baseline biopsy sample. The participants will include both male and female adults (age 18-50) who have been diagnosed with CD and have been on a gluten-free diet for at least two years with no other autoimmune diseases or medications.

The experiment will be a double-blind study with one half of all participants being unknowingly injected with hookworm after the three month diet probation period. There will be four groups: hookworm and gluten, hookworm no gluten, no hookworm and gluten, and no hookworm no gluten. All four groups will be given a pill unaware of what it is and a two day incubation period for the hookworm growth will be conducted. After the hookworm incubation the patients will begin their diets. All four groups will be given pre-prepared meals without knowing whether they are eating gluten. Every morning the patients will take a pill, containing iron for the patients with hookworm to avoid anemia.

This study will consist of three six week periods. Each six week period will consist of baseline duodenal biopsy first, the nematode positive or negative pill, the incubation, the gluten positive or negative diet, the after treatment duodenal biopsy, a course of either Albendazole or sugar pills, or rest. Both biopsies require one day for laxative treatment, one day for the procedure, and two days recovery. The nematode will require two days to incubate inside of the duodenum. The gluten diet will require only three days affecting the duodenum and blood work. The Albendazole requires two weeks to ensure the nematode has been killed. The rest of the six weeks will consist of a resting period with a controlled completely gluten-free diet. Those in the gluten positive groups will be on a wheat challenge and fed increasing concentrations of gluten for each of the three six weeks periods.

A duodenal biopsy will be taken after each three day gluten diet period, to monitor the effects on the duodenum. Blood samples will be taken from each patient once a day for the first three week portion of treatment in each of the three sets of testing. No blood tests will be taken during the duodenal biopsy or two week rest period. The blood will be tested for TG2, HLA, CD specific antibody, CD4, IL4, and IL10. After the three six week testing periods, all of the samples will be observed and compared. The histology from all of the biopsies will be qualitatively compared for relative thickness of mucous and length of villi. The blood samples will quantitatively compare the levels of TG2, HLA, CD specific antibody, CD4, IL4, and IL10.

If the results from the experimental gluten and *A. duodenale* group show positive results, the test will be continued for roughly two more weeks. Positive results would indicate no damage in the duodenum, elevated levels of *A. duodenale* secretions, and lowered levels of CD antibodies. These weeks would consist of three day gluten containing diet for half of the participants from the original group and a gluten free diet for the other half. A biopsy will be taken before and after the diet. Blood work will be taken from the first biopsy until the day of the last biopsy. The blood and tissue samples will be tested for the same

things as the first three sets of the experiment. If the tests still show elevated *A. duodenale* secretory products, decreased CD antibodies, and no damage to the duodenum, temporary *A. duodenale* infection could be considered the first non-dietary treatment for CD.

Significance

This proposed research will test whether or not infection with parasites can cure inflammatory immune responses in humans with autoimmune diseases. This research has the ability to further the treatment for autoimmune disease sufferers with the use of mutualism, or parasite and host benefitting from one another. If this study indicates that the temporary infection is indeed enough to cure the inflammatory immune response for a longer period of time, the next step would be the possibility of a vaccine. The idea of a hookworm vaccine has been discussed in regards to allergies, but this study would indicate that such a vaccine could cure a wider array of inflictions.

References

1. Behera, Bijayini, B. R. Mirdha, Govind K. Makharia, Shinjini Bhatnagar, Siddhartha Dattagupta, and J. C. Samantaray. "Parasites in Patients with Malabsorption Syndrome: A Clinical Study in Children and Adults." Digestive Diseases and Sciences (2007): 672-79. Print.
2. Daveson, A. James, Dianne M. Jones, Soraya Gaze, Henry McSorley, Andrew Clouston, Andrew Pascoe, Sharon Cooke, Richard Speare, Graeme A. Macdonald, Robert Anderson, James S. McCarthy, Alex Loukas, John Croese, and Lise Lotte Gluud. "Effect of Hookworm Infection on Wheat Challenge in Celiac Disease A Randomised Double-Blinded Placebo Controlled Trial." PLoS ONE (2011): E17366. Print.
3. Ferreira, I., D. Smyth, S. Gaze, A. Aziz, P. Giacomin, N. Ruyssers, D. Artis, T. Laha, S. Navarro, A. Loukas, and H. J. Mcsorley. "Hookworm Excretory/Secretory Products Induce Interleukin-4 (IL-4) IL-10 CD4 T Cell Responses and Suppress Pathology in a Mouse Model of Colitis." Infection and Immunity 81.6 (2013): 2104-111. Print.
4. Gaze, Soraya, Henry J. Mcsorley, James Daveson, Di Jones, Jeffrey M. Bethony, Luciana M. Oliveira, Richard Speare, James S. Mccarthy, Christian R. Engwerda, John Croese, Alex Loukas, and Richard Karl Grencis. "Characterising the Mucosal and Systemic Immune Responses to Experimental Human Hookworm Infection." PLoS Pathogens (2012): E1002520. Print.
5. Gujral, Naiyana. "Celiac Disease: Prevalence, Diagnosis, Pathogenesis and Treatment." World Journal of Gastroenterology 18.42 (2012): 6036-059. Print.
6. Hotez, Peter J., Simon Brooker, Jeffrey Bethony, Elena Bottazzi, Alex Loukas, and Shushua Xiao. "Hookworm Infection." New England Journal of Medicine 351.8 (2004): 799-806. Print.
7. Hotez, Peter J., David Diemert, Kristina M. Bacon, Coreen Beaumier, Jeffrey M. Bethony, Maria Elena Bottazzi, Simon Brooker, Artur Roberto Couto, Marcos Da Silva Freire, Akira Homma, Bruce Y. Lee, Alex Loukas, Marva Loblack, Carlos Medicis Morel, Rodrigo Correa

Oliveira, and Philip K. Russell. "The Human Hookworm Vaccine." Vaccine (2012): B227-232. Print.
8. Mcallister, Christopher S., and Martin F. Kagnoff. "The Immunopathogenesis of Celiac Disease Reveals Possible Therapies beyond the Gluten-free Diet." Seminars in Immunopathology 34 (2012): 581-600. Print.
9. Mcsorley, Henry J., Soraya Gaze, James Daveson, Dianne Jones, Robert P. Anderson, Andrew Clouston, Nathalie E. Ruyssers, Richard Speare, James S. Mccarthy, Christian R. Engwerda, John Croese, Alex Loukas, and Karol Sestak. "Suppression of Inflammatory Immune Responses in Celiac Disease by Experimental Hookworm Infection." PLoS ONE (2011): E24092. Print.
10. Schuppan, Detlef, Yvonne Junker, and Donatella Barisani. "Celiac Disease: From Pathogenesis To Novel Therapies." Gastroenterology 137.6 (2009): 1912-933. Print.
11. Sollid, L M, and K E A Lundin. "Diagnosis and Treatment of Celiac Disease." Mucosal Immunology 2.1 (2009): 3-7. Print.
12. "Albendazole: MedlinePlus Drug Information." U.S National Library of Medicine. U.S. National Library of Medicine, 2 Mar. 2015. Web. 7 Mar. 2015.
13. "American Gastroenterological Association - Advancing the Science and Practice of Gastroenterology." Preparing for an Upper GI Endoscopy; AGA. American Gastroenterological Association, 2015. Web. 2 Apr. 2015. http://www.gastro.org/patient-center/procedures/upper-gi-endoscopy.
14. Mcsorley, HJ, and A. Loukas. "The Immunology of Human Hookworm Infections." Wiley Online Library. Wiley, 3 Apr. 2010. Web. 2 Apr. 2015.