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Evaluating a Culprit: A Review of the Biochemical Mechanisms of Non-Celiac Gluten Intolerance

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The Biochemical Mechanisms of Non-Celiac Gluten Intolerance

Abstract

Non-Celiac Gluten Intolerance (NCGI) has evaded biological and chemical mechanisms since it was first reported in 1978. Celiac Disease, a multi-system immune mediated disorder has long served as the most similar counterpart to NCGI. Less cases of celiac disease are going undiagnosed reflecting an improvement on sensitive and decisive diagnostic tools. However, NCGI has been significantly growing as a diagnosis over the past decade with little match or similarity to the markers found in celiac disease patients. Critical evaluation of NCGI theories, experimentation, and existing hypotheses is necessary. This review seeks to synthesize multiple disciplines of gluten research and enhance public understanding of gluten through novel scientific communication initiatives.

Introduction

In 2013, 200 million restaurant visits within the United States included at least one gluten-free order (NPD Group). The number had grown nearly four times larger since the tracking began in 2009 and has continued to increase, with 11% of Americans adopting a Gluten-Free Diet (GFD) and 30% believing that gluten-free (GF) foods are inherently healthier (Lerner et al., 2019). However, estimates note that only around 1% of the U.S. population is diagnosed with celiac disease (CD), an adaptive immune system-mediated response that is marked by multiple signs of vicious enteropathy, or intestinal attack (Choung et al., 2016). The diagnostic tools for CD, both serological and histological, have seen continuous improvement, which may explain the four-fold increase in CD prevalence over the last 40 years (Oxentenko & Rubio-Tapia, 2019). Yet, the prevalence of those with non-celiac gluten intolerance (NCGI), or the

newer title (Schnedl et al., 2018), “people without CD avoiding gluten” (PWAG), has continued to rise (Choung et al., 2016).

Figure 1. An artistic rendering of the process of a gluten challenge. Subjects that aren’t eating gluten and are seeking a diagnosis are instructed to consume around 2 slices of bread each day for 2-6 weeks. This is because tests for gluten intolerance rely on prolonged gluten stress within the intestine to appear.

The confusion is understandable; many nutritionists, gastroenterologists, and prominent allergen researchers have stepped in to offer an opinion, yet the experimental data on NCGI and its comparison to CD is muddled and confusing. Top search articles claim that “Gluten is bad for your health” (*paleoleap.com*) in promotion of diets that are keto and paleo based. However, a recent study by Skodje et al. (2018) finds that a gluten challenge (Figure 1) within a double-blind, crossover trial caused no significant difference from placebo conditions, indicating gluten may not be the culprit at all (Skodje et al., 2018).

Stefano Guandalini, a pediatric gastroenterologist at University of Chicago Medical Center notes that, “If we did not know about the specific role of gluten in celiac disease, we would never have thought gluten was responsible for [NCGI]” (Servick, 2018). This skepticism

is supported by the consideration that we do not eat gluten in isolation often, and the culprit may be found among other less diagnosed intolerances such as fructan or lactose. The argument for the legitimacy of NCGI has bred skepticism in the science and the communication of gluten intolerance as good or bad depending on the opinion of the journalist. It appears that any medical condition that defies diagnoses has been noted as a gluten intolerance; however, further examination reveals that there are more stark differences.

This research aims to analyze the literature in context across fields of nutrition, biochemistry, and gastroenterology to understand the wider picture of NCGI and if gluten has truly been an offender within experimentally controlled studies. This review delves into the possible presence of undiagnosed intolerances, such as fructan or Fermentable Oligo-, Di-, Mono-saccharides and Polyols (FODMAPS), and expands upon the pertinence of looking into compounds such as histamine, an often overlooked but frequently intolerable compound to humans. Many reviews have shown that NCGI/Gluten Sensitivity (GS) is uncomfortable and causes significant symptomology both intestinally and extra-intestinally for those affected, but the serological and histological markers that lead to a CD diagnoses are repeatedly not present (Newnham, 2011; Biesiekierski et al., 2011; Leonard et al., 2017). This review aims to suggest a possible avenue of research that may lead to a more effective intervention than the elimination of gluten, which can potentially cause adverse health effects due to an absence of fiber, minerals and vitamins found in whole grains that reduce cardiovascular and Type 2 diabetes risk (Rosa-Sibakov, 2015). By thematically breaking down NCGI/GS and its comparison to CD as well as other intolerances through biochemical and structural properties, a more coherent direction for future proposed gluten intolerance is created. In accompaniment to the review, a novel evaluation of how to employ principles of scientific communication in order to disseminate the

pertinent narrative to a lay audience is provided. The novel idea of a "science influencer" is tested with this narrative and may encourage further analysis of social media techniques that can benefit translation of high-level research.

Current Trends

With the use of open-source data software, *Google Trends*, I have compiled the findings of the current state of gluten-understanding worldwide. Using the feature to analyze interest over time, which notes search interest relative to its peak popularity (100), I observed the difference in search trends for "Gluten-Free", "Celiac Disease" and "Gluten Intolerance" (*trends.google.com*). As predicted, "Gluten-free", the popularized term had significantly more searches over time and within every U.S. state (Figure 2). This visualization demonstrates a much higher interest in "Gluten-Free" as compared to associated intolerances.

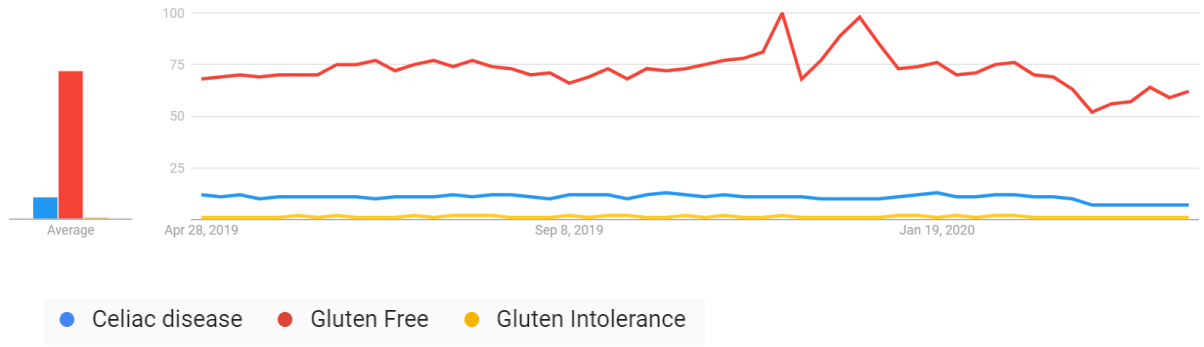

Figure 2. Google trends data from the U.S in the past 12 months indicating Interest over time for search terms "Celiac Disease" (blue), "Gluten Free" (red), and "Gluten Intolerance" (yellow). Interest over time represents search interest in reference to the highest popularity time point (100).

To visualize opinions of gluten as a good or bad food component, I analyzed the search queries "Is gluten good/bad?" and then to gauge interest, "why is gluten good/bad?". With the question that begins with "Is", one that begs a more direct answer, the interest in both varies but

"Is gluten good" is a question of higher popularity (Figure 3). For the query that adds a "why", the search trends remain variable but flip, so that "why is gluten bad" is of higher popularity (Figure 4). The trends indicate a want for a more direct answer on if gluten is good or bad for you, perhaps with an inherent preference for a positive result. The trend also shows an increase in a desire to know why it may be bad as opposed to lesser interest, if it is good for you.

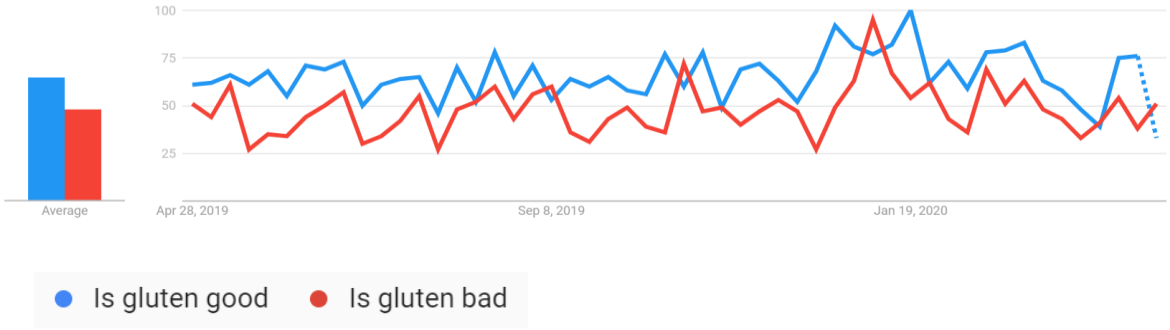

Figure 3. Google trends data from the U.S in the past 12 months indicating Interest over time for search terms "Is gluten good" (blue) and "Is gluten bad" (red).

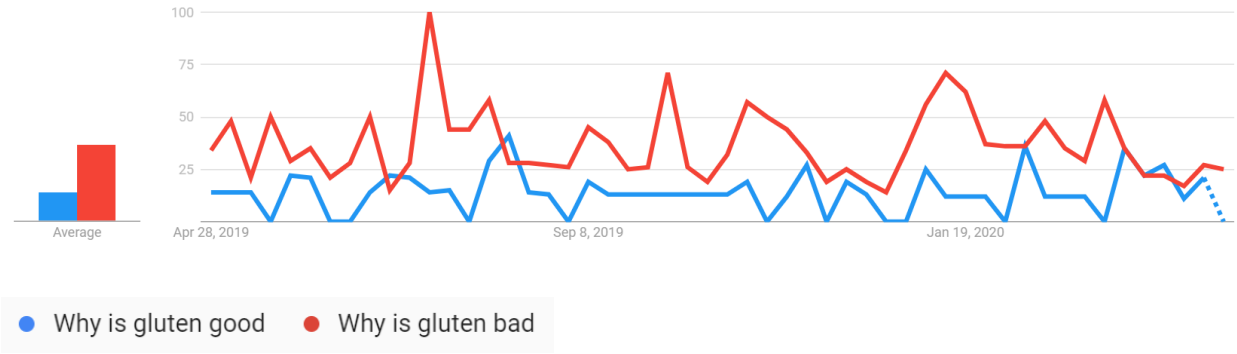

Figure 4. Google trends data from the U.S in the past 12 months indicating Interest over time for search terms "Why is gluten good" (blue) and "Why is gluten bad" (red).

This review will highlight the literature that may breed some confusion over whether an intolerance to gluten that is not celiac disease is either a bad or good, real or fake, and an unhealthy or healthy idea.

What is gluten?

To address the differences in NCGI and Celiac conditions appropriately, it is necessary to begin with the root of the offense and how it is chemically and biologically structured. Gluten makes up 85-90% of common wheat (*Triticum aestivium*) and is a main storage protein within the endosperm of the wheat kernel (Biesiekierski, 2017). It is a complex mixture of proteins that is often classified into gliadin and glutenin subgroups. The gliadin proteins are classified as prolamins and are monomeric proteins; originally intended for plant storage, they are insoluble in water and contain high levels of proline and glutamine residues that dominate in repetitive motifs. Glutenins belong to a glutelin subgroup that describes polymerized proteins which can be cleaved by temperature and various reducing and disaggregating agents (Scherf et al., 2016).

The protein matrix within the endosperm has a high allelic polymorphism and the variability of gluten proteins is greatly affected by environmental factors such as location, temperature, and fertilization. Notably, omega-5 gliadin, the major allergen for those with a traditional IgE mediated wheat allergy, is known to increase with growing conditions such as raised temperature and fertilization (Biesiekierski, 2017). The structural significance and variability within components of the wheat bran and germ is important in discussing nutritional properties. The matrix structure within the aleurone layer of the wheat bran holds the highest number of bioactive compounds and variation in the cell wall matrix affects the accessibility of necessary nutrients such as arabinoxylans and β-glucans.

However, the structure of gliadins within the gluten matrix is the most physiologically relevant when discussing the interaction of gluten and human digestion. Upon digestion of wheat, the gliadin peptides are resistant to gastric, pancreatic, and intestinal proteolytic enzymes (Mótyán et al., 2013). In the case of gliadin, which is riddled with proline, glutamine, and

hydrophobic residues, it remains resistant to degradation by trypsin, pepsin, and chymotrypsin, which are prohibited from cleaving the protein due to the overwhelming presence of such rigid residues (Biesiekierski, 2017). Since gliadin and glutenin peptides enter the small intestine with little degradation, they are quicker to trigger the immune system and certain pathways connecting the contents of the small intestine to the mucosal layers. These pathways include intestinal permeability induced by stress (marked by poor diet, exercise or binge drinking) or genetic predisposition. These open a transcellular pathway to allow gluten peptides across the epithelial barrier into the lamina propria (Lerner et al., 2019). A gliadin peptide beyond the epithelial barrier of the intestines has clear and validated immune effects in CD patients but less certain biomarkers and response within NCGS/NCGI patients (Bardella et al., 2016).

NCGI vs. Celiac

In Celiac Disease, the entrance of a gliadin peptide can trigger both an innate and adaptive immune response. Within typical gastrointestinal tracts, gliadin fragments often bind to chemokine receptors where they are often subsequently released (Leonard et al., 2017). However, a genetic predisposition to gluten sensitivity can prime an innate inflammatory cytokine response, and specific to CD patients, the gliadin fragment is transported across the intraepithelial barrier via the transcellular pathway (Leonard et al., 2017). The HLA DQ2 or HLA DQ8 gene is one of the first hints at a predisposition for celiac disease and is an introductory diagnostic marker (Oxentenko & Rubio-Tapia, 2019). In celiac patients, the gliadin fragments that reach the lamina propria are deamidated by tissue transglutaminase and then bind to the positive antigen presenting HLA-DQ2/DQ8 cells (Lerner et al., 2019). These cells respond as macrophages which signal the CD4 T-cell response that results in overproduction of B-lymphocytes, which release CD specific antibodies and noticeable amounts of intraepithelial

lymphocytes (Lenoard et al, 2017). These antibodies are of the IgA type since they regulate mucosal secretion and are specific to CD antigens such as tissue transglutaminase, gliadin, and deamidated gliadin peptide (Rashid & Lee, 2016).

The result of the immune cascade has villainous symptomology for the intestinal tract. Intraepithelial lymphocytes (IEL), a resulting product of the immune response, serve as a key defense mechanism at the intestinal barrier (Sheridan & Lefrançois, 2010). Yet, in CD the IEL count can be so high that it is more inflammatory than protective. Common histologic diagnosis of CD through duodenal biopsy reveals chronic inflammation of the lamina propria, villous atrophy, and increased intraepithelial lymphocytes (Oxentenko & Rubio-Tapia, 2019). Other obvious markers are the blood counts of CD specific IgA antibodies. Within a normal patient, the IgA anti-tissue transglutaminase antibody should be less than 4 U/ml and CD patients commonly have a concentration of greater than 40 U/mL (Rashid & Lee, 2016). With the use of serum for serologic testing, biopsies, and a genetic predisposition, there are multiple tools that can indicate obvious elevated symptomology within CD patients (Figure 5).

Table 1.

Serologic tests for celiac disease

ANTIGEN	ANTIBODY TYPE	TEST	SENSITIVITY, % (RANGE)	SPECIFICITY, % (RANGE)
Gliadin	IgA	ELISA	85 (57–100)	90 (47–94)
	IgG	ELISA	80 (42–100)	80 (50–94)
Endomysium	IgA	IFA	95 (86–100)	99 (97–100)
	IgG	IFA	80 (70–90)	97 (95–100)
Tissue transglutaminase	IgA	ELISA	98 (78–100)	98 (90–100)
	IgG	ELISA	70 (45–95)	95 (94–100)
Deamidated gliadin peptide	IgA	ELISA	88 (74–100)	90 (80–95)
	IgG	ELISA	80 (70–95)	98 (95–100)

ELISA—enzyme-linked immunosorbent assay, IgA—immunoglobulin A, IgG—immunoglobulin G, IFA—immunofluorescence assay.

Data from Leffler and Schuppan.[15]

Figure 5. Common antibody assays and specificity ranges within celiac disease.

NCGI patient diagnosis has not been so obviously rooted in biological signatures. The first marked case of NCGI was documented in 1978: a woman with symptoms that were not strong enough to resemble celiac was placed on a GFD to prevent “loose motions”, which ceased in four days. Yet, after a gluten challenge the IEL count and other testing was normal (Ellis & Linaker, 1978). This first encounter describes many other double-blind crossover and randomized studies that have attempted to reach the biological basis of NCGI over the past decade. Within a challenge trial, results showed that participants proved abnormal intestinal symptomology with significant difference but no CD anti-IgA antibodies, HLA DQ2/8 gene, or inflammation was found and seen (Biesiekierski et al., 2011). A review on NCGI studies noted that these experiments do not find dose dependent or gluten specific effects and the major biochemical tests reveal normal antibodies and villi structure (Bardella et al., 2016). With

significant symptoms that are extraintestinal (slowed thinking, memory, fatigue) and intestinal (abdominal pain, bloating, bowel abnormality) and no biomarkers to assist in classifying these changes many researchers indicate the need to look at other intolerances that may often go unnoticed (Gibson, 2020). Since NCGI has only been defined by symptoms that improve with withdrawal from gluten, it is pertinent to observe other compounds present in or around gluten-containing foods and how these may biochemically impact the body in a different way than celiac.

Fructan and FODMAPS

As mentioned in the introduction, fructan-containing compounds have been a primary proposal for intolerance since they are present in wheat and many other gluten-containing foods. In a randomized, double-blind, controlled study, participants were given placebo, fructan, or gluten containing meal bars for seven days. After the challenge, patients in the fructan group reported gastrointestinal symptoms significantly more than the placebo and gluten challenged group (Skodje et al., 2018).

Fructans are branched sugars containing fructosyl units with β(2-1) and β(2-6) linkages (Fedewa & Rao, 2014). Fructans are part of a commonly known class of nondigestible, but fermentable group of carbohydrates entitled FODMAPS (Fermentable Oligo-, Di-, Mono-saccharides And Polyols) which are often recommended as the component to reduce in patients with irritable bowel syndrome, ulcerative colitis, and other inflammatory gastrointestinal conditions (Eswaran et al., 2013). These compounds do not trigger any immune response but do result in fermentation and gas within the intestine. This process is both normal and healthy as the fermentation encourages positive bacterial growth that can strengthen the gut (Roager et al., 2019).

Some research has indicated that patients with unexplained GI symptoms who were negative for other conditions showed significant malabsorption of fructans. The intolerance can be due to a lack of enzymes that hydrolyze glycosidic linkages, therefore allowing more fructans to ferment in the colon (Fedewa & Rao, 2014). As FODMAPS are involved in multiple other GI conditions, there is stronger evidence that malabsorption of a compound other than gluten may better suit symptoms for certain NCGI patients. Yet NCGI has become marked by its presence of extraintestinal symptoms as well, making it elusive and unique to research with fructan. Fructan only affects distention of the abdomen, since it is only processed within the intestine (Verbeke, 2018). Therefore, although it may explain some elusive cases of NCGI, not all cases that affect extraintestinal and intestinal symptoms can be explained by fructan ingestion.

Amylase Trypsin Inhibitors

With the increase of modern wheat and insistent wheat cultivation in the United States (Schnedl et al., 2018), the properties and presence of amylase trypsin inhibitors (ATIs) has been put forth as a negative compound found alongside gluten in wheat, that is not gluten. ATIs are proteins that protect plants from pests and parasites by inhibiting digestive enzymes. Due to this property, there is a positive correlation for high yield and pest resistance with higher amounts of ATIs within the endosperm (Verbeke, 2018). They have been found to increase inflammation through activation of a toll-like receptor 4 pathway that releases cytokines in celiac and non-celiac patients (Junker et al., 2012). Since this is activation of an innate pathway, the initial impacts on the immune system line up closely to gluten's effect. However, little research has delved into whether there are truly more ATIs within modern wheat and if the common amount of 2-4% of protein (Biesiekierski, 2017) within wheat is enough to induce a significant reaction to the scale and pervasive nature of NCGI.

Histamine Intolerance

A less studied and promising avenue of research explores the idea that non-celiac gluten intolerance may closely match histamine intolerance. As first proposed in the journal of the European Histamine Research society (Schnedl et al., 2018), the intolerance of histamine can result in intestinal and extraintestinal symptomology and is a more targeted explanation than the current NCGI theories (Schnedl et al., 2018). Histamine is a biogenic amine that can appear in foods at variant levels depending on ripeness, cultivation and processing (Maintz & Novak, 2007). Fermented foods such as chocolate, cheese, and wine are often high in histamine content. Although gluten foods are not a direct source of histamine, histamine foods such as tomatoes are often eaten alongside gluten foods, like pizza or sandwiches. Interestingly, yeast is not a direct source of histamine but can propel histamine generation within the body (Schnedl et al., 2018). Histamine is normally broken down by amine oxidases upon ingestion that can decrease its toxicity, so some research has proposed that a lower level of diamine oxidase enzyme (DAO) could be a novel pathway to examine in NCGI (Schnedl et al., 2019)

Histamine intolerance (HIT) is a dysbiosis between the amount of accumulated histamine in the system and the ability for degradation (Maintz & Novak, 2007). The ability to degrade is caused mainly by a genetic or acquired impairment of the DAO enzyme. DAO expression is highly variable in the gut and the gene has been characterized by multiple single nucleotide polymorphisms (SNPs) that can greatly vary expression. Due to the variability in expression, HIT has been characterized by symptoms of a multifaceted nature mostly within the small bowel and epithelial tissues as this is where DAO is commonly found (Biegański, 1983). Along with the variability of expression and symptoms, SNPs within the DAO gene have been shown to be

strongly associated with inflammation in common GI conditions such as Crohn's Disease, ulcerative colitis, and other food allergies (Petersen et al., 2005).

This aligns more closely to other food intolerances than celiac. For example, nutrient intolerances such as fructan and lactose are marked by malabsorption due to a lack of current support in the body. With fructose intolerance, glucose transporters are not able to transport effectively and with lactose intolerance, there is less lactase that can appropriately break the compound down (Fedewa & Rao, 2014). In these instances, the intolerance is defined by a lack of function. However, the current perspectives on NCGI attempt to diagnose the intolerance with positive symptoms, such as higher antibody counts and inflammation, that is characteristic of autoimmune conditions, but not food intolerances.

Scientific Communication

The question of how far-reaching the understanding of histamine, outside of seasonal allergies, exists is little. Recent statements by both the American Chemical Society and American Association for the Advancement of Science note the need for increased competence and warmth, or trustworthiness, when explaining concepts to a non-scientific audience (Brownwell et al., 2013). It has become the assumption and norm that experts within their field are also experts at communicating, when experts have been so removed from hearing a complex term for the first time that every day “jargon” may include the use of “intraepithelial lymphocytes” and “IgA

antibodies".

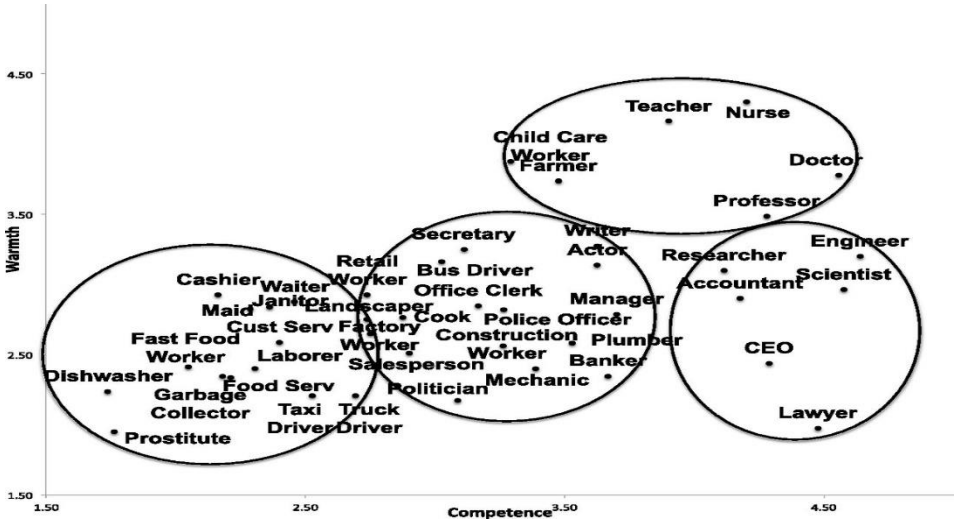

Figure 6. Findings from Fiske & Dupree (2014). Warmth–competence ratings of common jobs. Adults completed an online survey rating common jobs on their public images of being warm and trustworthy, as well as competent and capable. The middle cluster ranks relatively neutral on both measures of warmth and competence.

Accommodating language is only a first step when considering solid scientific communication. In Figure 6, A survey of multiple adults shows that researchers and scientists may be ranked highly in competence but fail to score as "warm" which directly affects how a lay audience may perceive someone as truthful. Notably, those within a high competence and low warmth cluster are often defined as "enviable" and create dislike and distrust (Fiske & Dupree, 2014). Although the goal of a scientist is to persuade, the best way to do so may be through warmth increasing techniques such as discussion, sharing and teaching.

"Sharing" as a concept is the most underutilized of these principles. Although many researchers and scientists are warming to the notion of communication, social media has been mistakenly viewed as a "pinboard" for accomplishments (Smith, 2016). The integral connection between social media and gaining warmth is consistent engagement (Illingworth & Prokop,

2017). An interaction with the audience lends boost to research, warmth and a knowledgeable public.

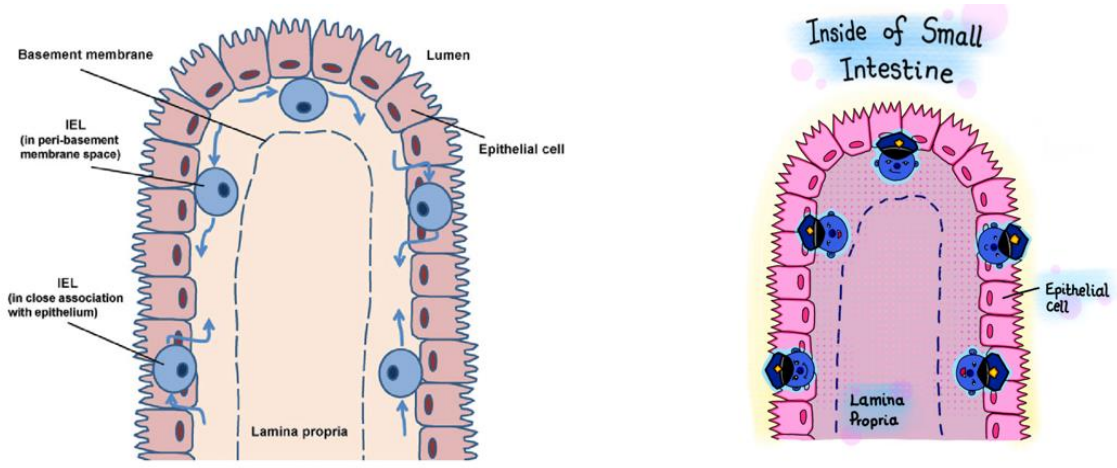

Figure 7. A published figure, *left*, (Sumida, 2019) examining the placements of intraepithelial lymphocytes. *Right,* a simplified but not compromised artistic rendering (Molly Yon Hin) of the location and function of intraepithelial lymphocytes.

To disseminate information through social media, I have begun to document both the NCGI narrative and other scientific food myths using a blog and widely accessible Instagram page. The Instagram profile has retained a steady presence of 255 followers with average impressions of posts around 400 (*Instagram analytics*). As demonstrated in Figure 7 and in Figure 1, the blog transforms difficult concepts to artistically simple illustrations that engage and excite, as well as inform.

Although the engagement data is rudimentary, the use of social media in a planned manner lends credence to new ideas for social media. The ability to explain immunological cascades does not only assist in communicating a passion but can increase accessibility of science to the public. It is often that results famously get miscommunicated or overhyped (Rinaldi, 2012), so future research and engagement should focus on interactive ways to showcase a body of research as opposed to discovery findings. In the case of NCGI, the amount of

misinformation about carbs and increase in patients reporting the ketogenic flu (Pogozelski et al., 2005).

Conclusion

Both gluten and histamine intolerances are fairly under-researched in their connection to one another. It is pertinent that the collaboration of fields outside the normal are brought in to bridge what could be the current nebulous mystery of non-celiac gluten intolerance. However, something that begs consistent and dedicated attention is the enhancement of communication surrounding food intolerances. As noted in the *Google Trends* data, little is known about what can be good about foods that are touted consistently as "What to cut out". A population that is using elimination diets for health can suffer from a lack of fiber, as the U.S. already sees (Roager et al., 2019) and other pertinent nutrients within whole grains that reduce diabetes, cardiovascular, and even gastrointestinal risk.

The narrative of NCGI tells a story of miscommunication and lack of collaboration. An Australian physician jokes that "any chronic medical disease should be regarded as celiac until proven otherwise" (Enders et al., 2015). This thinking has shifted not only the science, but the communication to the public. Public knowledge of gluten should not end at wheat, for the portrayal of bread to the public has been officially tainted. Celiac disease as well as diagnosed conditions should be taken seriously as well as nebulous conditions such as NCGI that may still be searching for their explanation. However, the need to understand intolerances aside from those that are mainstream (gluten) could be beneficial in providing patients with an easier treatment, such as an antihistamine, instead of a never-ending elimination diet.

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