Nonresponsive Celiac Disease Treated with a Unique Functional Medical Approach

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Abstract

A 16-year-old boy with nonresponsive celiac disease (NRCD), dermatitis herpetiformis, short stature, and failure to thrive, presented to this Functional Medicine practitioner because he had exceedingly high tissue transglutaminase (tTG) antibodies and poor growth, despite 10 months on a meticulous gluten-free diet (GFD). Immunological testing showed elevated antibody production against multiple peptides of wheat, food antigens, intestinal barrier dysfunction, lipopolysaccharide (LPS) antibodies, and polyreactive autoimmune reactions. An elimination diet, nutraceutical protocols to modulate the microbiome, address intestinal permeability, lower inflammation, and remove underlying bacterial infection were initiated. Global anti-inflammatory lifestyle modifications were recommended. Within 3 months of treatment, the patient’s tTG antibodies decreased by 14% for the first time since strict gluten elimination. Within 15 months, tTG IgG antibodies were nearly normal at 1.61 (0.03-1.60, ELISA Index). Test results improved dramatically in tandem with clinical progress. On a GFD and after initiating and maintaining these dietary and lifestyle changes, he gained 12 inches and 40 pounds. To our knowledge, this is the first published case of complete reversal of NRCD and failure to thrive by addressing endotoxin and lifestyle outside of a GFD.

Keywords: celiac disease, Nonresponsive Celiac Disease (NRCD), wheat related disorder, tissue transglutaminase, intestinal permeability, environmental enteric dysfunction, polyreactive antibodies, phospholipid antibodies, gluten contamination elimination diet, functional medicine

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1. Introduction

Celiac Disease (CD), wheat allergy, and non-celiac gluten sensitivity (NCGS) have been included under the umbrella term of ‘Wheat-Related Disorders (WRD)’. WRD can present with dramatically different clinical presentations from one patient to another [1]. Characterizing wheat and/or gluten sensitivity as primarily a disease of the gut is a historical misconception [2]. For every adverse reaction to wheat presenting with GI symptoms, there are 8 presenting without GI symptoms [3]. The impact of a WRD (with or without celiac disease) can manifest in any organ or system [4,5] and has been shown to impact autoimmune diseases [5,6,11], cardiovascular disease [7,8,9], malignant neoplasms [10], chronic inflammatory demyelinating neuropathy [12,13], autonomic neuropathy [13,14], mononeuritis multiplex [15], ataxia [5,13], cognitive impairment [16,17,18], schizophrenia [17,19], connective tissue diseases [20], allergies [21], inflammatory bowel disease [22,23], nephritis and nephropathy [24,25], hypercoagulation [26], thyroiditis [27,28], bone diseases [29], arthralgia and arthritis [30,31], various nutritional deficiencies [32,33,34] and even infections [35,36,37,38]. Adverse reactions to wheat have expanded to include CD (affecting approximately 1 in 100 in the general population) [39], wheat allergy (affecting approximately 1 in 1000 in the general population) [40], NCGS, and a category of sensitivities referred to as WRD, which includes wheat germ agglutinin sensitivity (a carbohydrate-binding lectin of wheat that also functions as a natural pesticide) [41,42], glutenomorphins sensitivity (an opiate receptor agonist protein in wheat) [43], exorphan and benzodiazepine sensitivity [44,45], a sensitivity to the family of amylase-trypsin inhibitors (ATIs) [46], and FODMAP sensitivity (fermentable oligo-, di-, mono- saccharides and polyols) [47]. (Figure 1).

If not properly harvested and stored, wheat may also be infested with fungi such as aspergillus and fusarium, leading to consumption of harmful mycotoxins [46]. Intriguingly, depending on the study, up to 46% of those diagnosed with a WRD, and not necessarily CD, with no current indicators of an autoimmune disease, demonstrate elevated ANA antibodies. Within 3 years, 87% of this group will receive a diagnosis of an autoimmune disease [48].
1.1. Functional Medicine

The functional medicine model is an individualized, patient-centered, science-based approach that empowers patients and practitioners to work together to address the underlying causes of disease and promote optimal wellness. It requires a detailed understanding of each patient’s genetic, biochemical, and lifestyle factors and leverages that data to direct personalized treatment plans that lead to improved patient outcomes.

By addressing root cause, in addition to symptoms, practitioners become oriented to identifying the complexity of disease. They may find one condition has many different causes and, likewise, one cause may result in many different conditions. As a result, functional medicine treatment targets the specific manifestations of disease in each individual.

1.2. Celiac Disease

Gluten is a storage protein found in most grains. In CD, the Triticeae tribe of gluten and glutenin proteins, including wheat, barley, and rye, trigger an immune response activated by dendritic cells via Toll-like Receptor (TLR) 4 in the proximal part of the small intestine. This leads to an inflammatory spectrum recognized first as small intestinal inflammation which then progresses through a destructive process (Marsh Classification system 0-IIIa, b, c), to eventual total villous atrophy. CD is characterized by villous atrophy and positive serology for immunoglobulin A (IgA) antibodies to tissue transglutaminase (tTG), neo-tTG, deaminated gliadin peptide, and/or endomysium [49].

The classical presentation of CD is diarrhea, steatorrhea, and weight loss, mainly reflecting the damage to the intestines [50]. However it is more recently recognized as a multi-system disorder, diversely affecting the brain and nervous system, musculoskeletal system, cardiovascular system, skin, liver and others [4,5,6,50].

Advances in diagnostic testing have yielded specific and sensitive serological tests for the detection of CD [49,51], including anti-IgA tissue transglutaminase, IgA anti-endomysial antibody, and IgG + IGA anti-deaminated gliadin peptide antibodies [49,50,52]. IgA tTG2 antibody is the most sensitive and specific serological marker of total villous atrophy CD [52]. Recent guidelines released by the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition state that high anti-tTG2 (10-fold or greater) antibody titers in symptomatic children is sufficient for diagnosis (without endoscopic biopsy) [53]. In adults, a positive IgA tTG2 is predictive of villous atrophy, however, the indications for endoscopic biopsies is still debatable between the various professional gastroenterological societies [54,55].

Common symptoms seen in pediatric cases of CD are gastrointestinal symptoms, dermatitis herpetiformis, dental enamel defects, osteoporosis, failure to thrive, short stature, delayed puberty, and iron deficiency anemia [56]. The present patient presented with gastrointestinal symptoms, dermatitis herpetiformis, delayed skeletal development, short stature, delayed puberty, and low testosterone.

1.3. Nonresponsive Celiac Disease

Nonresponsive Celiac Disease is defined by a limited initial response to a prescribed gluten-free diet (GFD), or the recurrence of symptoms despite maintenance of strict GFD in a patient who responded initially to GFD. The exact prevalence of NRCD is unknown but the presence of symptoms after treatment with a GFD is common in patients with CD. This clinical problem requires a systematic diagnostic and therapeutic approach because of the many distinct underlying etiologies. What are the expected clinical, serological, and histological responses after treatment with a GFD? The amount of time to feel better on a GFD is different for every person but clinical improvement is usually evident within weeks after treatment with a GFD. CD-specific serology normalizes by 6-12 months in most patients with strict adherence to GFD. Repeat upper endoscopy with intestinal biopsy may help to differentiate causes of NRCD associated with...
persistent mucosal damage from those usually associated with normal duodenal mucosa. However, full mucosal recovery after strict GFD is rare (8%) and could explain certain cases of NRCD. Out of 7648 celiac patients who underwent follow-up biopsy, persistent villus atrophy was present in 3317 (43%) [57]. All but 8% of those who regenerated the microvilli, still demonstrate excess inflammation and intestinal permeability. And in the majority of cases villous recovery is incomplete, especially in adult-onset CD [58].

Continued gluten exposure, whether intended or unintended, could also explain certain cases of NRCD. Gluten immunogenic peptides (GIPs), including the 33-mer peptide from alpha-gliadin, are resistant to luminal digestion [59,60]. Because of this resistance, GIP can be detected in faeces or urine and thus provides direct evidence and likely quantitation of gluten intake. The majority (85.7%) of children with CD under 3 years of age had faeces negative for GIPs. However, among those aged ≥13 years, faecal positivity for GIPs rose to 39.2%, thus identifying insufficient dietary education and/or non-compliance as a primary problem in recovery from CD. More males were positive for GIPs in faeces compared with females (60% vs 31.5%, P=0.034). Continued gluten exposure does not always affect serological markers. Serum IgA tTG2 antibodies were negative in 40 of the 56 patients with GIP-positive stools. Interestingly and to complicate the topic, IgA-tTG has numerous false positive and negative results [61,62].

If gluten contamination has been reasonably excluded via negative stool or urine analysis for GIPs, other additional disorders associated with NRCD must be actively investigated (Table 1) [63]. A subgroup of patients with NRCD may have more than one disorder associated with persistent symptoms (e.g., concurrent microscopic colitis and bacterial overgrowth). Thus, for these patients, specific treatment for all associated disorders is necessary to alleviate symptoms. This turned out to be the case with our patient [58,63].

| Table 1. Differential Diagnosis of Nonresponsive Celiac Disease Modified from Dewar et. Al [63] |
|---------------------------------------------------------------|
| **Diagnosis** |
| Continued dietary gluten exposure | Anorexia nervosa |
| Microscopic colitis | Exocrine pancreatic insufficiency |
| Small intestine bacterial overgrowth | Diverticular disease |
| Exocrine pancreatic insufficiency | Medication-induced diarrhea |
| Functional bowel disorders | Combined variable immunodeficiency |
| Protein-losing enteropathy | Human immunodeficiency virus |
| Lactose and fructose sensitivity or intolerance | Colorectal cancer |
| Cross-Reactivity with other food sensitivities | Anorectal dysfunction |
| Gardiasis | Incorrect diagnosis of celiac disease |
| Malignancies | Irritable bowel syndrome |
| Refractory celiac disease | Type I RCD |
| Type II RCD | Inflammatory colitis |

The present case study highlights a young man with suppressed growth and extremely elevated tTG2 antibodies, even after both a GFD and Gluten Contamination Elimination Diet (GCED). His chronic abdominal pain and his failure to thrive were very concerning to him and his family, as it held him back from his future dreams of becoming a competitive collegiate wrestler. After detecting widespread wheat peptide and food reactivity, microbial dysbiosis, intestinal permeability, translocation of microbial products, and polyreactive autoimmune suppression of growth. After 15 months of treatment his clinical symptoms and his test results improved in tandem.

### 2. Clinical History

A 16-year-old-boy (‘LAN’ for the purposes of this case) presented to the managing clinician with a previous diagnosis of CD, dermatitis herpetiformis (DH), severely elevated tissue transglutaminase antibodies (> 50-fold above reference range), despite 9+ months on a strict GFD, and failure to grow.

Dermatitis herpetiformis is a chronic, itchy, blistery skin rash experienced by 10 percent of people with CD. LAN had been treated by an endocrinologist for short stature, late puberty, and failure to thrive for approximately a year and a half. At 15 years of age, 4’8”, weight 90 lbs., LAN was the shortest person in his class. His stature percentile for age was below the 3rd percentile. For weight percentile based on age, he fell at 1.1% (CDC Children Growth Chart Calculator) [64]. He also complained of daily gas and occasional stomach pain. He was finally diagnosed with CD by endoscopy 10 months prior to visiting this clinician. LAN’s endoscopy indicated full villous atrophy and was visibly smooth in the images. At the time of his celiac diagnosis, LAN’s anti-tTG2 IgA was beyond quantitation and greater than 150 (normal < 3 U/mL, >10 U/mL is positive). In symptomatic children, a tTG2 antibody >100 is sufficient evidence of CD [65] and LAN’s blood levels far exceeded that. LAN had been following a strict GFD since that time. After 3 months on a strict GFD, his parents reported his DH had gone into complete remission and his insulin-like growth factor 1 (IGF-1), had gone from 165 to 284 ng/mL (an improvement of 72% in this short time). IGF-1 reflects improvement, his pediatric gastroenterologist said, suggested that LAN’s nutritional/calorie absorption had improved and he had a normal growth hormone axis. His tTG2 remained above the upper limit (>150 U/mL). His testosterone, which had been below 300, increased to 473 ng/dL (300-1,000 ng/dL), appropriate for an adult male.

LAN immediately started to grow. He experienced a growth spurt of 3 ½ inches (8.89 centimeters) and an increase of 2 shoe sizes over the following four months. However, LAN’s growth had plateaued at 5’2” and 95 pounds. He still had clinically abnormal growth, ranking at 1.8 percentile for stature compared to other adolescents his age. He was at 0.7% for weight based on age [64]. His parents, determined to leave no stone unturned, had their son assessed and treated at world-renowned Celiac
Research Centers, in both Maryland and Chicago. LAN’s pediatric gastroenterologist was surprised that LAN’s serum tTG2 antibody levels continued to be high after learning of the rigorous gluten-free standards in their home. Nonetheless, she told the family that LAN must be getting exposed to gluten and that was why the tTG2 antibodies remained high. The family was skeptical that there was any gluten in the home, but implemented an even stricter GCED, referred to as ‘The Fasano Diet’ [66]. After a further 6 months on the GCED, tTG2 antibody level was registered at 150. Thus 9+ months on a strict GFD had initiated positive results, but had plateaued with dangerously high tTG2 antibodies.

LAN and his parents were very concerned about his failure to grow. LAN excelled in academics and sports despite his size and health issues. He had a goal of qualifying for the wrestling team at college within the next few years. His short stature prevented him from being competitive. His mother reported, “He desperately wants to grow.” A bone age study confirmed failure to thrive demonstrating that LAN was 2 - 2 ½ years behind his actual age. LAN initially felt much better and more energy on the GFD. However, he still complained of stomach pain after eating and abdominal gas. LAN felt socially isolated because of the restricted diet and because he could no longer eat out at restaurants. With 70% of adults with CD demonstrating social phobias [67], and CD diagnosed in childhood associated with a 40% increased risk for suicide [68], the critical nature of identifying the underlying mechanisms thwarting this young man’s growth could not be overemphasized.

The family took extensive measures to remove all gluten from LAN’s diet after learning of his diagnosis. LAN’s father also had CD and his mother and aunt had wheat/gluten sensitivity. They were a strictly gluten free home and did not eat outside of the home. They bought all new pots, pans, and utensils. The mother reported that they were also very knowledgeable about cross-contamination. Recognizing that persistently elevated tTG2 antibody levels are a risk factor for numerous serious conditions, including lymphoma, LAN’s mother wanted help calming down his gut inflammation. She knew the essential nature of lowering his tTG2 antibodies, which at that time, measured at 50-fold above acceptable limits, and had not responded to 10 months on a GFD.

LAN’s mother reported an uneventful, healthy pregnancy and a natural childbirth with LAN that did not require medical intervention. LAN was breastfed from birth. He experienced reflux as a baby and had recurring ear infections from 2 to 5 years old, which required multiple rounds of antibiotics and surgery inserting drainage tubes in his ears at 5 years of age. There is a direct association between antibiotic usage in childhood and the development of CD [69]. He had all routine vaccinations as recommended by his pediatrician. LAN had no other allergies to foods, drugs, or inhalants. LAN’s mother had an IBS diagnosis and was negative for CD (genetic testing). She was on a specific carbohydrate diet and avoided gluten. LAN’s father had an elevated tTG2 antibody level and was positive for one celiac gene. He was also on a GFD. LAN’s two brothers were negative for both tTG2 antibodies and celiac HLA genes.

3. Diagnostic Assessment

Initial diagnostic testing included a bone age study, endoscopy, and blood testing for tTG2 antibodies, testosterone, and IGF1. LAN’s skeletal age assessment (SAA) [70] was 2 - 2 ½ years behind his actual age. He received his celiac diagnosis based on an endoscopy carried out by his pediatric gastroenterologist along with tTG2 antibody levels beyond laboratory identification limits. At the time of his celiac diagnosis, LAN’s tTG2 IgA was greater than 150. After 6 months on a GFD, the level was recorded at 150 and after a total of 9 months on a strict GCED, his tTG2 antibody level was still registering at 150 (reference range <3). The following laboratory investigations were recommended: contents of his intestinal microbiome, his immune reactions to multiple peptides of wheat, wheat-related cross-reactive foods, pathogenic intestinal permeability, and immune reactions against self-tissues (autoimmune reactions). Blood and stool were collected shortly thereafter.

Figure 2. Intestinal Antigenic Permeability Screen test results. This panel measures antibodies against proteins important in barrier integrity. Reference ranges are calculated based on the mean ± 2 standard deviations (SD). Results > 1 SD, and < 2 SDs above the mean, are considered to be equivocal. An equivocal result represents the range between negative and suspicious low positive results. Results >2 SDs are considered out of range, and positive. Actomyosin IgA results were obtained utilizing the INOVA Diagnostics Inc. QUANTA LITE Actin IgA kit. <20 units is considered a negative result. 20.1-24.9 units is an equivocal result and > 25 units is a positive result.
LAN’s intestinal antigenic permeability screen revealed elevated antibody production against occludin/zonulin IgG and IgM. He also showed high-normal or equivocal antibody reactions against actomyosin IgA and lipopolysaccharide (LPS) IgM. These results suggest that LAN was experiencing pathogenic intestinal permeability, LPS migration, and an immune response to endotoxin.

4. Testing for a Wheat Related Disorder

When testing serology for CD or a WRD, the most common blood test screens for antibodies to the 33mer peptide, alpha-gliadin. But the wheat kernel is comprised of hundreds of potentially antigenic protein components, many of which are associated with the pathogenesis of WRDs, with or without the enteropathy CD. Numerous studies recognize the amylase-trypsin inhibitors, the glutens, gliadins, and glutenin families of proteins in wheat, rye, and barley as primary, but not exclusive antigenic triggers of WRD [47,54,71,72,73,74].

Antibodies to alpha-gliadin, the 33mer peptide of poorly digested wheat was the first peptide to be screened for, but is only one of these potentially antigenic protein fragments. Historically, laboratories could only screen for elevated antibodies to this one peptide of poorly-digested wheat. Advancements in laboratory medicine allowed for the screening of LAN for 10 peptides of poorly-digested wheat, both IgA and IgG.

LAN’s antibody response to numerous peptides of incompletely digested wheat revealed significant activation of his immune response. He had extremely elevated antibodies to wheat, native & deamidated gliadin, alpha-gliadin, gamma-gliadin, glutenin, glutenomorphin-prodynorphin, gliadin-transglutaminase complex, and transglutaminase 2. Of note, his tTG2 IgG antibodies were > 3-fold higher than the range (0.3-1.6). Positive serology to transglutaminase 3 and 6 confirmed a systemic autoimmune reaction beyond the gut. This is consistent with LAN’s earlier diagnosis of Dermatitis Herpetiformis and CD. However, although meticulous, complete gluten removal from the diet was successful in putting his Dermatitis Herpetiformis into remission, these actions were unsuccessful in lowering his antibody responses to multiple peptides of poorly digested wheat to normal range.

Serum was drawn to examine an immune response to proteins in foods other than toxic-gluten containing foods, many being common in gluten-free diets (GFD), some of which may be cross-reactive with wheat (Figure 4).

LAN was highly reactive to 17 out of 24 foods on the cross-reactive foods panel. This high reactivity to multiple presumably “healthy” foods has been associated with both immune activation secondary to macromolecular transport into systemic circulation via pathogenic intestinal permeability of incompletely digested foods [75], and with a Cross-Reactivity or Molecular Mimicry mechanism [76]. Thus, multiple positive antigens on this panel strongly suggests an underlying pathogenic intestinal permeability [77]. Many of these whole foods are naturally gluten-free and LAN was eating them on a daily basis, such as dairy, corn, potato, and egg.

In parallel, serum was drawn to examine an immune response to 24 different tissues of self (an auto-immune mechanism) (Figure 5).
**Figure 4.** Gluten-Associated Cross-Reactive Foods and Food Sensitivity measures immune reactivity to proteins in foods. This panel measures both immunoglobulin G and immunoglobulin A antibodies in serum. Results that are “Out of Range” are 2 Standard Deviations (SD) from the mean. Equivocal results are 1 SD greater than the mean, suggesting a high-normal result that might be clinically relevant.

**Figure 5.** Multiple Autoimmune Reactivity Screen. This serum panel measures IgG and IgA antibody production against self-proteins. High antibody reactions may be a sign of activated autoimmune mechanisms beyond the range of normal. Reference ranges are calculated based on the mean ± 2 standard deviations (SD). Results > 1 SD, and < 2 SDs above the mean, are considered to be equivocal. An equivocal result represents the range between negative and suspicious low positive results. Results >2 SDs are considered out of range, and positive.
LAN’s autoimmune reactivity screen suggested that his immune system was targeting a plethora of his own tissues, including proteins involved in stomach, thyroid, adrenal, testis, pancreas, liver, platelet, heart, phospholipids, cytoskeleton microfilaments, neuronal proteins and brain. This laboratory panel is used to detect subclinical autoimmune mechanisms before they develop into full-blown autoimmune diseases. Indeed, tissue antibodies can show up in the bloodstream up to 30 years before the clinical threshold of disease has been reached [78,79,80,81].

LAN was instructed to collect a stool sample on 3 consecutive mornings with the Day 3 sample being a purged (diarrhea induced) sample (Figure 6).

LAN’s stool test showed that he had very low beneficial colonic microbiota, especially Lactobacillus species, E. coli, and Enterococcus species. Essentially Normal = 4+. He was negative for dysbiotic flora, parasites, and fungi. Secretory immunoglobulin A (sIgA) was low-normal at 58.3 (range 51-204 mg/dL). sIgA is an immune defender in the gut mucosal lining that binds, neutralizes, and helps to remove pathogens, toxins, and allergens [82]. A low-normal sIgA in a 16-year-old male suggests that LAN had weak gut immune defenses. Lysozyme was extremely elevated at 877 (range <=600 ng/mL), indicating an inflammatory state. Lysozyme is a neutrophil derived enzyme that helps to destroy Gram-positive bacterial cell walls, is high in inflammatory and non-inflammatory bowel diseases [83], and appears to be an objective parameter of the inflammatory activity of IBD [84]. Other measures of inflammation were normal and markers of digestion were normal.

![Comprehensive Stool Analysis / Parasitology x3](image)

**Figure 6.** LAN’s initial comprehensive digestive stool analysis

5. Clinical Findings and Preliminary Working Diagnosis

LAN had evidence of pathogenic intestinal permeability. LAN’s elevated LPS antibodies suggested a possible underlying bacterial and/or viral infection (Figure 2). Lipopolysaccharides are structural components of the Gram-negative bacterial cell wall. They are potent triggers of the inflammatory response and LPS antibodies can be used to study major bacterial infections in serum [85].

The managing clinician found that LAN was highly reactive to numerous wheat and gluten proteins (Figure 3). These antibodies persisted despite meticulous removal of gluten and strict avoidance of cross-contamination from the diet and from the entire home. In addition to peptides of poorly-digested wheat, LAN had high reactivities to many other foods (Figure 4).
Further, the clinician hypothesized that the elevated tTG2 antibodies were not due to gluten exposure but instead due to infection or microbial endotoxin. The physiological role of tTG2 is not restricted to celiac disease. tTG2 antibody has been proposed to be an indicator of infectious disease in non-celiac children [86].

Summary of Investigative Findings
- Dangerously elevated tTG2 antibody levels, unresponsive to a strict GFD
- Severe sensitivity to multiple peptides of wheat
- Celiac Disease-Associated Autoimmune Endocrinopathies [87]
- Severe intestinal permeability
- Immune reactions to multiple foods, some severe
- An Autoimmune Polyendocrine Mechanism
- LPS infiltration into systemic circulation
- Markers of intestinal inflammation
- Increased endotoxin levels

LAN had been diagnosed with two autoimmune diseases fueled by a sensitivity to wheat - CD and DH. Elevated biomarkers of reactivity to multiple tissues (Figure 5) suggested to the clinician polyreactive antibodies to self and numerous underlying autoimmune mechanisms at work. (see Discussion)

The practitioner suspected that LAN’s failure to thrive and persistently elevated tTG2 antibodies with polyreactive antibodies and autoimmune mechanisms was secondary to a systemic inflammatory response fueled by endotoxin infiltration.

LAN’s growth and regenerative cellular mechanisms were likely being suppressed by a highly inflamed systemic inflammatory response, which did not manifest with overt pathology beyond CD and DH.

The clinician’s working diagnosis for LAN was: non-responsive celiac disease with protective severely elevated tissue transglutaminase and polyreactive autoimmune suppression of growth, secondary to systemic inflammation.

6. Therapeutic Intervention

The clinician recommended an elimination, rainbow diet and protocols to reduce intestinal permeability. Nutraceuticals to reduce inflammatory proteins, modulate inflammation, and address a suspected underlying bacterial or viral infection were prescribed. The patient complied with all protocols for two years and dietary recommendations transitioned into lifestyle habits (an essential component of success in the clinician’s view, which was communicated to the patient).

Diet. LAN was directed to continue on a strict gluten-free, sugar-free, dairy-free diet. All 17 foods that were highly reactive on the cross-reactive foods profile (Figure 4), were removed from his diet. The clinician referred the family to a functional medicine nutritionist and coach who gave them gluten-free, dairy-free, and sugar-free guidance, recipes, and encouragement with a strong emphasis on brightly colored vegetables and fruit that were customized for LAN’s food restrictions [88].

He ate a 100% organic, meats grass-fed, whole foods diet and rotated a variety of proteins, organ meats including liver, heart, wild caught fish, and organic chicken. Wild meats such as bison and elk, were also introduced.

Intestinal barrier repair. In addition to eliminating allergenic foods, the managing clinician advised the patient to reduce intestinal permeability by focusing on creating an anti-inflammatory environment both in the gut and systemically.

Studies of human gene-related inflammation suggest that, of the approximately 25,000 human genes, approximately 5%, or some 1200 genes, are involved in inflammation [89]. The clinician’s approach centered around the concept of a pleiotropic approach [90] to modulate both as many of the activated inflammatory genes and the dormant anti-inflammatory genes as possible. From this perspective the nutritional recommendations included:
- a once-per-day ingestion of a packet containing 6 tablets/capsules containing 22 nutrients daily, GS Support Packs (NuMedica, Inc.)
- colostrum - GS Immuno Restore (NuMedica, Inc.), three times a day in a smoothie to include organic blueberries (or other high polyphenol fruits) and turmeric.
- L-Glutamine - GS L-Glutamine Powder (NuMedica, Inc.) was given in the morning away from food.
- Vitamin D - GS Vitamin D-3 (NuMedica, Inc.)

Nutrition. LAN was given nutrients to support tissue healing and immune function. Besides one capsule of 5000 IU vitamin D/day in his ‘pack’, LAN was recommended one sublingual drop (1,200 IU/drop) per day of vitamin D3 as cholecalciferol in mycellized form. The clinician’s logic of sublingual utilization was to increase systemic levels vs the tablet of Vitamin D likely being utilized predominantly in the intestines. Later, after serum Vitamin D testing, sublingual dose was increased to two drops to raise his vitamin D levels to a target serum level of 75-100 ng/ml. As oral intake of ribose in humans has been shown to lead to an enhanced resynthesis of ATP [91], essential to growth, powdered Vitamin C with Ribose at 4000 mg of vitamin C and 2000 mg of ribose, Vitality C (American Nutraceuticals, Inc.) was recommended to be included in his daily smoothies.

Underlying bacterial and endotoxin accumulation. The clinician prescribed Biocidin (BioBotanical Research, Inc.), an antimicrobial herbal formula containing a Proprietary Herbal Blend of Bilberry extract, Noni extract, Milk Thistle, Echinacea Purpurea extract, Echinacea Angustifolia, Goldenseal, Shiitake extract, White Willow Bark, Garlic, Grapeseed extract, Black Walnut (hull and leaf), Raspberry, Fumitory extract, Gentian, Tea Tree oil, Galbanum oil, Lavender oil, and Oregano oil. The clinician’s goal was to address suspected underlying bacterial/viral infiltration and accumulation. Delivery was recommended in capsule, spray and drop format.

The clinician was fooled by LAN’s visual level of health and the initial antimicrobial recommendations in all 3 delivery systems was overwhelming to his system. LAN did not respond well to the initial dosing, which the clinician suspected was a Jarisch-Herxheimer reaction. The Herxheimer reaction is an adverse ‘die-off reaction’ from antibiotic treatment, first discovered with the treatment of syphilis [92]. Symptoms, such as fever, chills, and skin rashes, worsen temporarily due to the release of
A mold inspection of the home was negative. Electromagnetic excess testing of their home was negative, but the family still reduced electromagnetic pollution by turning off their router when it was not in use and in the evenings. They used low electromagnetic frequency light bulbs and didn’t use smart meters on their home for water or electric. In terms of water purification, they used a whole house chlorine water filter and used a reverse osmosis (RO) filtration system for all drinking and cooking water with compensatory extra mineral supplementation to accommodate RO filtration of minerals/metals. They installed out of house range hoods that ran when gas appliances were used. Dishwashers were run only in the evening while the family slept [97]. They used low-chemical or no-chemical cleaning supplies and body care products. They stopped using Teflon or non-stick pots and pans. They installed MERV 15 (antimicrobial) air filters in the pans. They installed MERV 15 (antimicrobial) air filters in the home. LAN also saw a chiropractor for musculoskeletal evaluation and treatment.

7. Clinical Follow-up

After approximately 9 months on the diet and supplementation protocol, LAN’s tTG2 antibodies, for the first time since instituting a GFD, had decreased to 129 U/mL. This represented a 14% decrease from the upper limit of quantitation (>150 U/mL). LAN’s vitamin D was suboptimum at 41 ng/ml and his dose was increased. He was exercising on a regular basis. At 5’4” and 126 lbs, LAN’s height placed him at the 22.3 percentile, when compared to his age-matched peers [64]. He had gained 2 pounds of muscle and was 10 pounds heavier. LAN’s stools were formed and normal most of the time. He didn’t complain of stomach pain or discomfort any longer, but had occasional gas. The parents noted that LAN had “flaming cheeks” and softer stools when he ate nuts and excluded those from his diet.

In August 2013, approximately 11 months into the protocol, LAN was doing quite well. His testosterone increased to 890 ng/dL (300 – 1,000 ng/dL). Stools continued to be normal. LAN no longer complained of stomach problems, though he reported occasional gas.

Per the clinician’s recommendation, LAN had been working with a Pololquin- Certified personal trainer in preparation to wrestle in the winter.

They were continuing LAN’s gut healing protocol in the hopes that he could handle college life and the long list of cross-reactive foods that he would surely encounter. At this point, LAN’s symptoms had improved, his growth had returned, energy levels were better, and his tTG2 antibody levels were decreasing (but still more than 40-fold above normal <3).

8. Follow-up Testing

Approximately 15 months after the initial tests were ordered, a second round of testing was ordered on LAN. His blood specimens were received at Cyrex Laboratory and results were reported seven days thereafter.
LAN’s wheat reactivity test results improved significantly so that 13 of 15 previous abnormal markers of wheat and gluten protein reactions were within normal parameters. Gamma gliadin 15mer IgG reduced by greater than 50% (3.49 in the first test) to just Out of Range levels. And critically important, tTG2 IgG antibody (originally > 3-fold above acceptable limits), was now close to below out of range levels.
LAN’s cross-reactive food reactions improved markedly but some still remained Out of Range. He was still highly reactive to cow’s milk, whey protein, chocolate milk, oats, corn, and potato. Even though he was highly reactive to 6 foods on follow-up testing, all but one of these biomarkers (corn) had improved significantly.

This was in marked contrast to his high reactivity to 17 foods on the first test. He showed equivocal reactivity to five foods. One can assume that LAN’s improvements were supported by his strict avoidance of the foods which he was highly reactive to on the prior test, as directed by the practitioner.

**Figure 9. Follow-up Cyrex Array 2 (Intestinal Antigenic Permeability Screen) test results**

LAN’s follow-up testing showed that his intestinal barrier was stronger. He no longer had any findings out of range, but occludin/zonulin IgG and occludin/zonulin IgM (which were extremely Out of Range previously at 4.48 and 4.94 respectively), decreased to the equivocal range (1.29 and 2.08 respectively). Statistically, LAN’s initial results were greater than 3-fold above 2 standard deviations from the mean and his follow-up results were Equivocal (one standard deviation from the mean). He was equivocally positive for occludin/zonulin IgG antibodies, suggesting either a continued reduction in immune activation or past immune activation with current vigilance [98] and equivocally positive for occludin/zonulin IgM antibodies, suggesting a continuing immune recognition of freshly damaged and/or senescent cells, often via oxidation-associated neo-determinants [99].

Actomyosin IgA antibodies (originally at 22.67) were within normal range. This is suggestive of elimination of the autoimmune mechanism against the actomyosin network of the intestinal lining. However, Occludin/ Zonulin IgG and IgM’s equivocal readings suggested some ongoing reactivity. His intestinal barrier therefore continued to require focused attention. The clinician explained that a microbiome that had developed over 16 years does not change to a totally anti-inflammatory healthy, diverse microbiome in a few months. With the inescapable threat to chemical exposures on a daily basis, which contributes to intestinal dysbiosis [100,101] continued vigilance was required.

**Figure 10. Follow-up Cyrex Array 5 Multiple Autoimmune Reactivity Screen**
LAN’s follow-up autoimmune reactivity screen showed dramatic improvements across the board. In his test 15 months earlier, LAN was out of range for 14 different self-tissues. All of those reactions were absent from the follow-up test as well as all equivocal findings.

In correlation with his improved tTG2 levels, LAN appeared to have quieted down the spectrum of co-morbid autoimmune mechanisms previously identified, as all of his self-biomarkers were within normal range.

LAN’s follow-up stool test revealed improved beneficial microbiota, reduced lysozyme levels, and increased secretory IgA – all hallmarks of a less-inflamed intestinal microbiome environment. Commensal microbes had increased (2+ Bacteroides fragilis group, 2+ Bifidobacterium spp., 2+ Lactobacillus spp., 1+ Clostridium spp.). However, no growth of E. coli or Enterococcus spp. was detected. Commensal (imbalanced) microbiota were found at low levels (Alpha hemolytic strep, Enterobacter cloacae, Pseudomonas aeruginosa, Staphylococcus aureus were all 1+ growth).

LAN’s sIgA, originally ‘anemic normal’ for a 16-year-old boy at 58.3 mg/dl improved to a healthy 151 mg/dL (51-204 mg/dL). Lysozyme reduced, originally 46% above normal at 877 ng/ml to 13% above normal at 679 ng/mL (<= 600 ng/mL). Yeast and parasites were negative. Fecal pH was high and occult blood was detected. Digestion and inflammatory markers were within normal ranges.

9. Recent Clinical Follow-up

As of the writing of this case, LAN was healthy and well. By the time he was 20 years old, LAN was at the 28.3 percentile for height and 7.5 percentile for weight. At 23 years old, LAN was 5’8” and weighed 130 lbs. On a GFD only, LAN grew 3 inches and gained 5 pounds. After beginning treatment with this clinician, LAN grew an additional 6 inches and 35 pounds (Table 2). LAN was able to achieve his athletic goals, however he did not return to high school wrestling because of concerns about higher cortisol levels, triggered by wrestling, and its consequences. Instead, he was a starter on the soccer team and worked with a trainer for some time. He went on to successfully complete college and get married.

As of this writing, LAN was still on the strict elimination diet. His parents attributed much of his improvement to the cross-reactive foods that were removed from his diet. LAN reported no gut symptoms at the present time and said he had been free of gut symptoms since the dietary changes. His parents noted that his hair texture was thicker and silkier, his energy levels were better, and his sleep had improved. They also remarked that his skin was better, especially since he continued to be free of Dermatitis Herpatiformis.
Table 2. Age, Height, Weight, and Percentile Ranking Over the Course of Treatment for a Boy with Nonresponsive Celiac Disease Short Stature, and Underlying Autoimmune Mechanisms

| Date       | Age          | Height | Height (in.) | Weight | Stature for age percentile [64] | Weight for age percentile [64] |
|------------|--------------|--------|--------------|--------|-------------------------------|-------------------------------|
| 10/15/2011 | 15 yr 3 mo   | 4’8”   | 56”          | 90 lbs | 0%                            | 1.1%                          |
|            | Initiated gluten free diet |        |               |        |                               |                               |
| 07/27/2012 | 16 yr 2 mo   | 5’2”   | 62”          | 95 lbs | 1.8%                          | 0.7%                          |
|            | Initiated elimination diet, anti-inflammatory, nutritional, and lifestyle modifications |        |               |        |                               |                               |
| 04/23/2013 | 16 yr 9 mo   | 5’4”   | 64”          | 126 lbs| 4.5%                          | 22.3%                         |
| 04/13/2016 | 19 yr 10 mo  | 5’8”   | 68”          | 130 lbs| 28.3%                         | 7.5%                          |

10. Discussion

Globally, one out of four children under 5 years are affected by linear growth delay (stunting). This syndrome has severe long-term sequelae including increased risk of illness and mortality and delayed psychomotor development. Stunting is a syndrome that is linked to poor nutrition and repeated infections [102]. The pathophysiology of chronic infections includes the gateway of intestinal permeability, and among the several potential intestinal luminal stimuli that can stimulate zonulin release, small intestine exposure to large amounts of bacteria (bacteria overgrowth), endotoxin (LPS) and gluten, the protein causing WRDs, have been identified as the most powerful triggers. All three of these triggers activate a similar immune response in the proximal section of the small intestine [103], via TLR4.

Gluten is misinterpreted by the zonulin pathway as a potential harmful component of a microorganism [104]. The clinician emphasized to LAN that his body, and every other human’s body, responds to gluten as if it is a parasite and activates an inflammatory cascade via dendritic cells activating the receptor designed to protect us from parasitic disease, TLR4, with every exposure [105].

LAN was a 16-year-old boy who had Dermatitis Herpetiformis and NRCD. He presented to the managing clinician for persistent linear growth delay and elevated tTG2 antibodies (>150), even after maintaining a strict gluten protein reactivity, pathogenic intestinal permeability, showed dramatic improvement for wheat and gluten cross reactivities, and tTG2 antibodies were near-normal (0.03-1.6 Elisa Index). LAN’s intestinal permeability test results improved, suggesting that his immune system was no longer attacking his own tissues and that underlying autoimmune processes were arrested. LAN grew 8 inches over the course of the protocol and gained 31 pounds.

10.1. Environmental Enteric Dysfunction (EED)

Environmental enteric dysfunction (EED) refers to a subclinical disorder of intestinal function common in tropical countries and in settings of poverty, malnutrition and economic disadvantage [106]. The mechanisms and metabolic cascade of EED contributing to stunted growth has stunningly similar mechanisms and metabolic cascade to the Failure to Thrive in CD (Table 3).

Table 3. Similarities of Environmental Enteric Dysfunction and Celiac Disease

| Environmental Enteric Dysfunction (EED) | Celiac Disease (CD) |
|----------------------------------------|---------------------|
| EED is a syndrome producing stunted growth in children characterized by [107]: | CD is an autoimmune disease that can produce a Failure to Thrive characterized by: |
| Initiates innate and adaptive immune response | Initiates innate and adaptive immune response |
| Altered intestinal permeability/inflammation [108] | Altered intestinal permeability/inflammation [109] |
| Microbial translocation [110] | Microbial translocation [111] |
| Systemic inflammation | Systemic inflammation [112] |
| Endocrine dysfunction [113] | Endocrine dysfunction [114] |
| Suppressed IGF-1 [115] | Suppressed IGF-1 [116] |
| Secondary carnitine deficiency [117] | Secondary carnitine deficiency [118] |
| Abnormal fatty acid oxidation [119] | Abnormal fatty acid oxidation [119] |
| Alterations in polyphenol and aminoacid metabolites [117] | Alterations in polyphenol and aminoacid metabolites [120] |
| Metabolic dysregulation of sulfur amino acids, tryptophan, and the urea cycle [121] | Metabolic dysregulation of sulfur amino acids, tryptophan, and the urea cycle [122] |
| Anti-LPS antibodies [110] | Anti-LPS antibodies [123] |
Systemic inflammation (i.e., the release of proinflammatory cytokines and activation of the innate immune system) primarily associated with endotoxin overload and malnutrition [124,125,126] is considered an important pathway by which EED inhibits growth [127,128]. With a symptom ratio of 8:1 (extra-intestinal to intestinal symptoms), CD also has systemic inflammation as a primary pathway. In EED, microbial translocation due to altered barrier integrity drives intestinal inflammation, which further exacerbates gut dysfunction and promotes systemic inflammation. In turn, systemic inflammation can further perturb immune function and suppress the production of insulin-like growth factor I (IGF-I), creating a cycle of malnutrition, infection, and immune dysfunction [129,130,131]. CD has a very similar mechanism with systemic inflammation resulting from intestinal permeability and LPS infiltration, suppressing the production of insulin-like growth factor I (IGF-I) [129,130,131].

Another aspect to emphasize is the greater risk for those children who had height impairment in early childhood, possibly associated with EED, to present overweight and with obesity in adulthood when exposed to a high calorie diet, which has been called a ‘triple burden’ [121]. Of interest to note is that over 50% of adult celiacs were found to have a Body Mass Index (BMI) over 25 kg/m2 at diagnosis [132]. LAN presented with stunted growth, altered barrier integrity, evidence of microbial translocation, and suppressed IGF-I. Even though LAN came from an affluent U.S. family and was not suffering from a lack of quality food, the clinician suspected the dynamic of EED was at play here due to malabsorption and malnutrition from both innate and adaptive immune response to CD.

10.2. Polyreactive Autoimmune Suppression of Growth, Secondary to Systemic Inflammation

The managing clinician suspected a plausible mechanistic pathway: LAN had a globally inflamed systemic immune response suppressing his anabolic growth and regenerative cellular mechanisms [133]. Clinically, low-grade systemic inflammation is associated with markedly inhibited tissue protein synthesis and restricted linear growth [113].

Multiple, interrelated factors could lead to LAN’s ‘perfect storm’ and delayed development (Figure 12):

![Figure 12. The Failure to Thrive ‘Perfect Storm.’ A mechanistic hypothesis](image-url)
Gluten initiates transient intestinal permeability in all humans who consume it, not just in those with genetic vulnerability to CD. In LAN, gluten-triggered transient intestinal permeability was exacerbated by his genetic predisposition to CD. His CD genetic vulnerability initiated his innate immune response in the gut and inflammation early in his pathology. It would have also increased zonulin, a protein which regulates intestinal tight junctions, which led to intestinal permeability. Pathogenic intestinal permeability permits wheat peptides, LPS, and other large antigenic food molecules through the tight junctions, into the submucosa, setting off systemic immune reactions. Circulating LPS can then bind onto tissue which may have triggered a local increased tTG2, furthering inflammation. Transglutaminase 2 (tTG2) is a ubiquitous human cellular enzyme and high levels – along with antibodies to tTG2 can have far-reaching pathogenic effects in human tissues.

10.3. Celiac Disease and Growth Retardation

About 8–10% of pediatric patients are diagnosed with CD when they are investigated for causes of short stature, and 10–40% of pediatric patients have short stature when investigated for CD [135-140]. Approximately 19–59% of the non-endocrine causes of short stature are CD-dependent [141]. The cause of short stature is not completely understood in patients with CD. Based on growth retardation in CD, it is assumed that malabsorption and malnutrition of food intake caused by inflammation and histological damage in the mucosa of the small intestine contribute to this symptom.

However, LAN’s immune reactions to LPS and wheat lectins, and severe reactions to wheat gliomerphins and prodynorphins, with their known binding capacity to several tissues and organs [45,142], suggested to the clinician another possible catalytic mechanism in the polyreactive systemic autoimmune reaction.

Another identified mechanism of stunted growth in CD is a disrupted growth hormone/insulin-like growth factor-1 axis, participation of increasing levels of inflammatory markers in serum to IGF-system dysregulation, a decrease in IGF-1 and IGFBP3 levels, and autoimmune hypophysitis [138,139,143]. The final heights of patients with CD were inversely correlated with diagnosis age. The present patient demonstrated an additional mechanism-global endotoxin accumulation initiating a local protective tTG2 response with resulting local inflammation suppressing regenerative protein synthesis and stunted tissue growth.

10.4. Celiac Disease and Inflammation

Celiac Disease is an autoimmune disease characterized by autoantibody production (transglutaminase and/or endomysium), autoimmune enteropathy [50], and autoimmune comorbidities up to 30 times more prevalent than in the general population [144]. Antibiotics and reduced duration of breastfeeding have been shown to increase the risk of developing CD [145,146] and, as was the case with our patient, CD patients have altered microflora populations [145,147] and reduced Paneth cell numbers which continually stresses the epithelial lining in the GI tract. It is interesting to theorize that a reduced Paneth cell concentration in the intestinal epithelium, which is a hallmark of complicated CD [148], may have contributed to a compensatory increase in prevalence of polyreactive antibodies.

Peptides of wheat initiate transient intestinal permeability in all individuals [149]. This response to poorly-digested peptides have been recorded to occur within 5 minutes of wheat entering the proximal part of the small intestine [150]. Stimulation of cells with gliadin, in contrast with other tested food proteins, leads to enhanced expression of maturation markers (CD80, CD83, CD86, and HLA-DR molecules) and increased secretion of chemokines and cytokines (mainly IFN-gamma, IL-6, IL-8, IL-10, TNF-alpha, growth-related oncogene, MCP-1, MCP-2, macrophage-derived chemokine, and RANTES (CCL5) [151]. It is a mildly cytotoxic molecule to all humans, even for those without evidence of a gluten-related disorder [152,153]. At the cellular level, wheat rearranges the cytoskeleton, causes apoptosis, inhibits cell growth, changes the redox balance and enhances intestinal permeability by repeated zonulin activation, with corresponding production of zonulin antibodies, eventual increased antibody production [153,154], damaging tight junctions and the gastrointestinal lining [152,153].

Systemic damage due to an immune inflammatory cascade in CD may occur by a number of pathways. In the intestinal lamina propria, gliadin activates intestinal epithelial lymphocytes which release inflammatory cytokines beginning with IFN-gamma, IL-6 and TNF-alpha production follows. Additional macrophages and fibroblasts are recruited to the site leading to inflammation and destruction of the intestine villous tissue [52].

10.5. Tissue Transglutaminase

LAN presented to the clinician with a primary concern of dangerously elevated tTG2 antibodies and NRCD. Despite following both a GFD and an even more rigorous GCED, LAN’s tTG2 antibodies remained dangerously elevated, higher than the quantitative limit of the assay, at >150 (range <3 U/mL). The family was very well educated and took extensive measures to fully remove gluten from the home and the clinician therefore concluded that the family had effectively removed gluten from LAN’s diet. The question remained: Why was LAN’s tTG2 so difficult to decrease? What mechanism must be occurring for which the body was being activated to produce such high levels of tTG2 if it was not inadvertent exposure to wheat? Although tTG is considered a biomarker and an accurate serology indicator of total villous atrophy CD, its role is not limited to CD. In fact, it has a role in numerous human diseases (Table 4) and manifests with a broad spectrum of symptoms (Table 5) [17,25,29,30,31,153,155,156].
Table 4. Tissue Transglutaminase Involvement in Human Disease. Modified from Lerner, et al [17,25,29-31,153,155-156]

| Disease Type     | Specific Condition                                                                 |
|------------------|-------------------------------------------------------------------------------------|
| Inflammatory     | Rheumatoid arthritis, osteoarthritis, chronic kidney disease, cystic fibrosis, alcoholic steatohepatitis, allergic inflammation |
| Degenerative     | Senescent cataracts, functional aging of tissues                                     |
| Neurodegenerative| Huntington’s disease, Alzheimer’s, Parkinson’s disease                               |
| Malignant        | Ovarian, pancreatic, breast, melanoma, lung cancers and glioblastoma                |
| Metabolic        | Type 1 and 2 diabetes mellitus, Maturity-onset diabetes of the young (MODY), diabetic cardiomyopathy, glycerolaldehyde 3-phosphate dehydrogenase, alpha-ketoglutarate, phosphoglycerate dehydrogenase, fatty acid synthase deficiencies |
| Autoimmune       | Type 1 diabetes, Celiac disease, Dermatitis herpetiformis, multiple sclerosis, SLE, bullous pemphigoid, Sjögren’s syndrome, rheumatoid arthritis |
| Reproductive     | Fetal failure to thrive, pregnancy stabilization                                     |
| Genetic          | Elliptocytosis, Ehlers-Danlos syndrome type III, harlequin ichthyosis, ichthyosis bullosa, glucagon deficiency, pachyonychia congenital, α ketoglutarate dehydrogenase deficiency, phosphoglycerate dehydrogenase deficiency, alkaptonuria, Huntington’s, recessive dystrophic epidermolysis bullosa, cystic fibrosis |

Studies suggest a role for infectious agents in the production of tTG2 antibodies. In one study, non-CD children with elevated tTG2 antibodies also had elevated antibodies to Epstein Barr virus and Coxsackie virus. Authors concluded, “During an infectious disease, anti-transglutaminase antibodies can be produced temporaril y and independently of gluten” [86].

tTG2 is expressed in multiple cell types, in both intracellular and extracellular compartments [155,156,17,158]. It is involved in a variety of cellular processes, including adhesion, migration, growth, survival, apoptosis, differentiation, exocytosis, wound healing, angiogenesis, autophagy, cyto-protection and extracellular matrix organization [158,159].

Table 5. Symptom Patterns Associated with Anti-tTG Antibodies. Modified from Lerner, et. al. [4,5,7,8,9,13,14,23,25-31]

| General            | Weakness, lassitude, malaise, weight loss, short stature, failure to thrive, celiac disease, non-celiac gluten sensitivity |
|--------------------|----------------------------------------------------------------------------------------------------------------------|
| Gastrointestinal   | Diarrhea/constipation, anorexia, nausea and vomiting, flatulence and abdominal distension, abdominal pain, motility disturbance, glossitis/apthous ulcers, celiac disease, non-celiac gluten sensitivity |
| Metabolic          | Anemia features, bleeding tendency, edema, cramps/tetany, dental enamel hyperplasia, malabsorption, malnutrition, hypox/hyperthyroid, celiac disease, non-celiac gluten sensitivity |
| Musculoskeletal     | Bone pain and fractures, myopathy, osteopenia, osteoporosis, celiac disease, non-celiac gluten sensitivity               |
| Neuropsychiatric   | Depression, anxiety, schizophrenia, cerebrospinal degeneration, ADFID, learning disorders, celiac disease, non-celiac gluten sensitivity |
| Neurological        | Hypotonia, developmental delay, headache, and cerebellar ataxia, paraesthesia, peripheral neuropathy, celiac disease, non-celiac gluten sensitivity |
| Reproductive       | Menstrual irregularities, recurrent miscarriages, abnormalities of sperm morphology and motility, infertility, inhibition of placental development and intrauterine growth retardation, celiac disease, non-celiac gluten sensitivity, wheat related disorders |
| Skin               | Variety of rashes, petechiae, acne, celiac disease, non-celiac gluten sensitivity                                        |

A primary function of the human immune system is to prevent the dissemination of microbes and the resulting bacteremia or sepsis. One of the less-recognized features of tTG is its immune function of pathogen entrapment [160]. As an early responder to massive infiltration of bacteria, tTG2 appears to be the dominant early immune innate response [160] Upon contact with hemolymph or blood, microbes are almost instantaneously targeted by tTG activity leading to formation of small aggregates and ultimately to sequestration by the clot matrix. The clinician theorized this ‘pathogen entrapment’ by tTG2 may be a contributing mechanism to LAN’s elevated tTG and prioritized an anti-bacterial protocol.

A second theorized mechanism elevating LAN’s tTG was in response to the damage occurring throughout his body from the 16 different elevated levels of antibodies to self - including stomach, thyroid, adrenal, testis, pancreas, liver, platelet, heart, phospholipids, cytoskeleton microfilaments, neuronal proteins and brain tissue. Elevated antibodies to self initiate an inflammatory mechanism in the target tissue causing tissue damage and an accelerated apoptotic state.

In normal conditions, unwanted cells within the body are removed by apoptosis, a process which culminates in apoptotic cell (AC) removal by professional phagocytes and antibodies with an accompanying anti-inflammatory response preventing systemic inflammatory disease and autoimmune conditions. Removal of AC is an integrated, multistep process that, in vivo, involves recruitment of macrophages. This is followed by recognition and binding of cell corpses prior to engulfment through the use of a range of receptors and soluble bridging molecules to bind dying cell ligands.

Tissue transglutaminase has been shown to play an important role in this process [161]. tTG plays a prominent role in AC engulfment by promoting phagocytic portal formation. It is also crucially important in immune modulation to prevent inflammation and the development of autoimmunity [162,163]. Given that the in vivo loss of tTG2 leads to delayed phagocytosis of ACs by macrophages and to development of autoimmunity [162], the clinician theorized that LANs elevated tTG antibody levels were an immune effort to avoid this delayed phagocytosis of ACs being created by the multiple autoimmune mechanisms in his body.

Apoptotic cells are generated by diverse physiological processes, ranging from the elimination of damaged (or precancerous) cells to deletion of cells during developmental morphogenesis [164]. The culmination of the apoptotic program is the phagocytosis of the AC. In mammals prompt removal of ACs is required to prevent the release of potential self-antigens and the onset of autoimmune-like syndromes [26,165,166]. Tg2−/− mice develop an age-dependent autoimmunity due to defective
in vivo clearance of apoptotic cells [163]. It was theorized that this young man’s body was unable to keep up with the apoptotic clearance process and had developed numerous autoimmune-like mechanisms (16 different elevated antibodies to self), inhibiting developmental morphogenesis (a Failure to Thrive). In this theorized scenario, the elevated tTG levels were representative of an innate immune response attempting to prevent the onset of autoimmune-like syndromes and/or precancerous cell formation.

With 16 different tissue antibodies elevated above acceptable ranges, it was likely that LAN’s apoptotic activity overwhelmed his growth activity thus contributing to a categorical ‘Failure to Thrive’. It was suspected that this accelerated cycle of cell death was fueled by LAN’s dysbiotic, pro-inflammatory microbiome, contributing to an ongoing pathogenic intestinal permeability, macromolecular food molecule infiltration, endotoxin infiltration, responding systemic inflammation and the proverbial ‘dog chasing its own tail’, merry-go-round.

Transglutaminase antibodies are considered a significant disease-modifying factor in neurodegenerative diseases because they may enzymatically stabilize aberrant aggregates of pathogenic proteins. tTG2 has been shown to play a major role in cancer genesis and metastatic progression and spreading. In some conditions tTG2 expression serves as a predictor of cancer behavior and prognosis or reactivity to specific drug therapies [155].

10.6. Intestinal Permeability

One critical function of epithelial-lined surfaces is to define the interface between separate body compartments. Examples include the skin, which maintains a barrier that supports overall homeostasis and prevents systemic infection, and the renal tubule, which forms a barrier that maintains gradients between the renal interstitium and the sterile tubular lumen to allow active and passive transport to regulate urine composition. The intestinal mucosa has a far more difficult charge: it must balance the needs for a barrier against a hostile environment, like the skin, with the necessity of active and passive transport, like the renal tubule. An intact intestinal barrier is, therefore, critical to normal physiological function and prevention of disease [167,168].

The intestinal epithelial lining forms a physical, chemical, and immunological barrier that separates the host from the outside world and "keeps the bugs at bay". If the permeability of the epithelial lining is compromised, it allows environmental toxins, food macromolecules, and bacteria from the lumen of the gut to enter the bloodstream and lymphatic circulation, leading to disruption of tissue homeostasis. Perturbation of the gut microbiota, together with increased intestinal permeability (or “leaky gut”) can activate an appropriate protective immune response, creating inflammation, and/or tissue damage [169].

The intestinal epithelium forms an efficient barrier to most undegraded food proteins or microorganisms but allows the sampling of the luminal contents by paracellular or transcellular pathways [170]. Intestinal permeability tests that measure absorption of inert probes have been used for at least 50 years to detect intestinal dysfunction. They are altered in most digestive diseases, and CD is no exception. Permeability to inert sugars is useful to test general intestinal dysfunction but does not directly measure permeability to gliadins or other food proteins [170,171] and therefore cannot help in the understanding of CD aetiology [172].

LAN had two diagnosed autoimmune diseases fueled by a sensitivity to wheat - CD and DH. Figure 2 showed intestinal permeability, LPS migration, and an immune reaction to LPS. In addition to severe sensitivity to peptides of wheat, the boy was highly reactive to 17 cross-reactive foods (Figure 4). His high reactivity to multiple foods suggested pathogenic intestinal permeability with macromolecular infiltration into systemic circulation activating a protective immune response to the antigen [75].

Microbial translocation and disturbed intestinal permeability may be a causative element behind autoimmune disorders [168,169]. The classical paradigm of autoimmune pathogenesis involving a specific genetic makeup and exposure to environmental triggers has been challenged by the addition of additional elements [173] including the loss of intestinal barrier function [174], an altered microbiome [134], and a systemic immune response. Recognition of this 5-factor dynamic in the etio-pathogenesis of autoimmune mechanisms provides an understanding whereby the autoimmune mechanism may be arrested if the interplay between genes and environmental triggers is prevented by re-establishing intestinal barrier function [175].

10.7. Lipopolysaccharides

Multiple lines of evidence pointed to a role for microbial, or endotoxin, involvement in the present case. LAN had LPS antibody levels 1 SD above normal, very high lysozyme, and persistently elevated tTG despite a GCED. He also showed intestinal permeability, CD, and autoimmunity, all of which can be initiated by microbial infection [174,176,177]. Further, LAN had what appeared to be a Herxheimer reaction when first treated with an antimicrobial herbal formula and he had to titrate up slowly.

LAN’s antibodies to LPS (Figure 2) led the treating clinician to suspect endotoxin accumulation. Lipopolysaccharides are structural components of the Gram-negative bacterial cell wall. Known as endotoxin, when found in the host, they are potent triggers of the inflammatory response. Detection and quantitation of LPS-antibodies is therefore a way to measure the immune response to bacterial/endotoxin infections in serum [85].

While LAN’s microbial dysbiosis was not excessive according to his stool test (Figure 6), it did show extremely high lysozyme, a marker of inflammation. Lysozyme is an enzyme produced from neutrophils which help destroy Gram-positive bacterial cell walls. It is high in both inflammatory and non-inflammatory bowel diseases [83].

The boy showed high phospholipid antibodies (Figure 5). Auto-antibodies that recognize phospholipids themselves are not associated with thrombosis, but with infectious diseases [178]. A growing body of evidence shows that antiphospholipid antibodies are not necessarily...
pathologic. Instead, they belong to the natural antibody repertoire, and likely represent a polyreactive mechanism secondary to endotoxin infiltration [179,180,181,182].

10.8. Polyreactive Antibodies

The ability of polyreactive antibodies to bind to a variety of different antigens suggests that these antibodies represent the humoral component of the innate immune system [17]. In fact, upon entering the host, pathogens will be exposed almost immediately to polyreactive antibodies since these antibodies are constantly present in saliva and serum. Hence, the initial encounter of polyreactive antibodies with pathogens could serve as a first line of defense and give the adaptive immune system time to be activated [183]. Polyreactive antibodies are an ancient part of the immune system and have been found in jawed vertebrates going back as far as the shark [184,185]. Polyreactive antibodies are considered part of the ‘nonspecific’ defenses of the body and react individually with unrelated epitopes. These natural auto-antibodies are found in higher concentration in Secretory IgA (sIgA) than in serum IgA and act as a first barrier to infection during the pre-immune activation period [186]. Polyreactive antibodies are a major contributor to the broad anti-bacterial activity of the natural antibody repertoire and bind to a variety of structurally unrelated antigens. They bind strongly to some bacteria (Streptococcus oralis J22, Streptococcus oralis 10557, Streptococcus mitis 15914 and E. coli BL21), moderately to other bacteria (Streptococcus oralis C104, Streptococcus gordonii 34, Actinomyces naeslundii T14V and E. coli K12) and weakly or not at all to still other bacteria (Streptococcus gordonii DL1, Actinomyces naeslundii 12104 and E. coli O157: H7). They can bind to and lyse bacteria such as Pseudomonas aeruginosa. They enhance phagocytosis, neutralize the functional activity of endotoxin (LPS), and are bactericidal. Recent evidence of scavenger receptor function reveals how the transition from natural and polyreactive antibody responses towards potentially more aggressive B cell activation occurs. Innate immune activation is crucial in defense against invading pathogens, including recognition by pattern recognition receptors, such as scavenger receptors. These receptors bind an array of modified self and foreign ligands and have, for this reason, the ability to regulate the immune response, including B cell activation. In this respect, increasing evidence suggests that these receptors are involved in autoimmune and might provide a link to autoimmune disease [179].

LAN initially showed high polyreactive antibodies which suggested systemic inflammation and underlying autoimmune processes, possibly inhibiting developmental morphogenesis (Failure to Thrive, Figure 12). After 15 months of treatment, LAN’s findings were dramatically improved. Dietary changes, especially food restrictions, gut healing treatments, and other interventions helped to lower inflammation and reduce the autoimmune mechanisms. He was no longer making excessive numbers of antibodies to his own tissues, such as stomach, thyroid, adrenal, testis, pancreas, liver, platelet, heart, cytoskeleton microfilaments, neuronal proteins and brain. It is possible that imminent autoimmune diseases, outside of CD, were averted by resolving the underlying polyreactive antibody response.

From a clinician’s perspective, LAN’s excessive autoimmune reaction results (Figure 5) can be overwhelming to understand in a 16-year-old fully functional young man and easily ignored or categorized as ‘false positive’ results. However, when for the sake of discussion, a clinician looks at the main presenting complaint (elevated tTG antibody levels and failure to thrive), these test results are a critical clue to potential underlying mechanisms. Tissue antibodies can show up in the bloodstream up to ten years before the clinical threshold of a diagnosable disease has been reached [78,79,80,81]. Polyreactive antibodies and autoimmune mechanisms were theorized to be contributing to LAN’s failure to thrive. LAN’s growth and regenerative cellular mechanisms were likely being suppressed by a highly inflamed systemic immune response, without an overt diagnosable disease outside of CD and DH.

10.9. Microbiome

Stool testing initially showed very low beneficial colonic microbiota, low-normal sIgA, and extremely high lysozyme (see Figure 6). The microbiome is known to be disturbed in CD [147]. And it is abnormal in intestinal permeability [169]. Together with the risk of an infectious agent, restoring microbial health in LAN’s enteric tract was of paramount importance. After treatment, beneficial gut bacteria and sIgA had increased. Lysozyme was much improved but remained high, indicating a continued neutrophil response to Gram-positive bacteria and potential inflammation in the gut.

11. Conclusions

Approximately 25% of the world’s children aged <5 years have stunted growth, which is associated with increased mortality, cognitive dysfunction, and loss of productivity [115]. The present case illustrates similar mechanisms of growth suppression between a 16-year-old young man from an affluent family in the U.S. and children in poverty in third world countries. This case study is an example of nonresponsive CD with growth suppression, despite a rigorous GCED failing to address accumulative underlying mechanisms. Laboratory testing revealed extremely elevated tTG and severe sensitivity to peptides of wheat. His testing also showed adverse reactions to 17 foods, severe intestinal permeability, and polyreactive antibodies which suggested underlying autoimmune processes at work and chronic inflammation. There were some markers of microbial triggers of disease, such as LPS antibodies, elevated lysozyme levels, and elevated tTG antibodies. The working hypothesis was that the patient had polyreactive autoimmune suppression of growth secondary to systemic inflammation.

A comprehensive treatment to modulate inflammation, exclude antigenic foods, reduce microbial translocation of LPS, boost nutrition, and create a healthier intestinal barrier led to a marked decrease in tTG activity, sustained weight gain, and increased growth of 8 inches. Test results
reflected a parallel improvement on the cellular and immunological level. Polyreactive antibodies against self-tissues returned to normal levels, suggesting that further autoimmune diseases may have been averted.

This case study demonstrates that NRCD may be the result of dysbiosis, intestinal permeability, microbial and LPS translocation into circulation, systemic inflammation, and immune activation. By addressing these areas of disturbance, the clinician was able to eventually reduce the inflammatory cascade, lower tTG antibodies, and promote growth and development in an ambitious, compliant young man.

**Abbreviations**

NRCD-nonresponsive celiac disease; tTG-tissue transglutaminase; LPS-lipopolysaccharide; CD-celiac disease; GFD-gluten-free diet, WRD-wheat-related disorders; ATIs-amylase-trypsin inhibitors; FODMAP- fermentable oligo-, di-, mono- saccharides and polyols; TLR-Toll-like Receptor; GIPs-Glutten immunogenic peptides; GCED-Gluten Contamination Elimination Diet; DH-dermatitis herpetiformis; SAA-skeletal age assessment; EED-Environmental Enteric Disturbance; AC- apoptotic cell; slgA- secretory IgA; MODY-Maturity-onset diabetes of the young.

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TO- Treating Physician, Conceptualization, Methodology, Data curation, writing the original draft. AL- Data curation, writing- review and editing, supervision.

**Informed Consent**

Informed consent from both the patient and his parents were obtained.

**Conflict of Interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

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