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WHEAT/GLUTEN PROTEOME REACTIVITY AND AUTOIMMUNITY™

OVERVIEW

MEASURING GLUTEN-REACTIVITY

Introduction

Current testing for Gluten-Reactivity and Celiac disease (CD) includes serum IgG and IgA against gliadin and tissue transglutaminase-2 (tTG2). These antibodies are measured against minor components of a wheat protein called alpha-gliadin. However, wheat consists of multiple proteins and peptides including, alpha-gliadin, omega-gliadin, glutenin, gluteomorphin, prodynorphin, and agglutinins. Any of these antigens has a capacity to challenge the immune system. Because of this heterogeneity of gluten proteins and peptides, multiple variations in T-cell responses may occur against them. Recent medical research indicates that a large number of gluten epitopes, may be implicated in the development of Gluten-Reactivity, CD and other associated conditions. The repertoire and hierarchy of gluten peptides stimulate the intestinal T-cells and results in a significant elevation of IgG and IgA production.

The measurement of IgA and IgG against multiple gluten epitopes in blood can have important implications in the accurate diagnosis and design of therapy for Gluten-Reactive and CD patients. For a comprehensive approach to this problem, pioneering, patent-pending technologies have been developed to measure IgA and IgG against various wheat components including alpha-gliadins, -17-mer and native + deamidated -33-mer, gamma-gliadin-15-mer, omega-gliadin-17-mer, glutenin-21-mer, opioid peptides (prodynorphin + gluteomorphin), gliadin-transglutaminase complex, and tissue transglutaminases -2, -3 and -6.

Research performed in-house confirms that different Gluten-Reactive and CD patients recognize an array of gluten antigens. For example, one patient reacts to omega-gliadin, but not to alpha-gliadin. The second patient reacts to all gliadin peptides, and the third patient reacts only to the wheat germ agglutinin.

Gluten-Reactivity is a systemic autoimmune disease with diverse manifestations. CD or gluten-sensitive enteropathy, is only one aspect of a range of possible manifestations of reactivity to gluten. And yet, this enteropathy, “one of the most common lifelong disorders in both the U.S. and Europe,” receives the lion's share of focus to the point of ignoring other manifestations. Autoimmune disease, the third leading cause of morbidity and mortality in the industrialized world, is 10 times more common in a gluten-sensitive enteropathy than in the general population. Thus, the burden on society from Gluten-Reactivity cannot be overestimated. Earlier identification might result in earlier treatment, better quality of life, and an improved prognosis for these patients.

The emphasis on Gluten-Sensitive Enteropathy (Celiac disease) as the main manifestation of Gluten-Reactivity has been questioned. It is now accepted that Gluten-Reactivity is a systemic illness that can manifest in a range of organ systems. Such manifestations can occur independently of the presence of the classic small-bowel lesion that defines CD. That Gluten-Reactivity is regarded as principally a disease of the small bowel is a historical misconception.
The Gluten-Reactivity has been proposed to include not only CD, but also Gluten-Reactive patients without mucosal lesions. From the skin (Dermatitis Herpetiformis, Psoriatic arthritis, Alopecia areata, Dermatomyositis, Cutaneous vasculitis), to the muscles (inflammatory myopathies), to the brain (Gluten Ataxia, altered neurotransmitter production, Schizophrenia, peripheral neuralgias, idiopathic neuropathies) and beyond, pathology to gluten exposure can occur in multiple systems without evidence of an enteropathy.

Negative serology should not necessarily reassure the clinician of neither negative immune activation nor pathology. Several reports show that in the majority of Celiac patients, antibodies to gliadin and transglutaminase may be negative. In particular, seronegative CD seems to be quite frequent in patients with milder intestinal damage (Marsh I-IIIa lesions). And these lesions often present without elevated Celiac markers. Some reports identify the sensitivity as low as 27-31% with lesser degrees of villous atrophy. Patients with non-Villous Atrophy Gluten-Reactivity (Marsh I, Marsh II) are more likely than others to test negative for tissue transglutaminase and endomysial antibodies. Despite the many published reports on seronegative Celiacs, this subgroup understandably continues to be forgotten or not included in diagnostic workup, unless presenting with Celiac crisis. Why would that be? This seronegativity is due to the measurement of antibody IgG and/or IgA against only one antigen of wheat, alpha-gliadin.

Numerous complications have arisen regarding an accurate identification and diagnosis of Gluten-Reactivity, with or without the enteropathy CD. Clinicians have been frustrated with the high percentage of false negative serology. For example, CD has been called the “Unforgiving Master of Non-Specificity and Disguise.” Therefore, if Gluten-Reactivity and CD go undetected for years, the results could be devastating autoimmune conditions. Mechanisms of action are shown in the Figure 1.

** Immunological Mechanisms Underlying Gluten-Reactivity and Intolerance

![Figure 1](image)

*Figure 1 – The mechanisms behind Gluten-Reactivity can ignite either Th-1 or Th-2 or a combination of both immune reactions.*

Therefore, should Healthcare Practitioners limit their diagnostic inquisitiveness solely to the well-referenced indicators of a severe gluten enteropathy (anti-transglutaminase and endomysial antibodies)? Numerous researchers suggest not.
Current serology testing—although highly sensitive and specific for severe gluten enteropathy—does not address the diversity of gluten peptides and the need for more sensitive markers of Gluten-Reactivity with or without CD.

**GLUTEN PEPTIDES**

During digestion, gluten proteins are enzymatically broken down in the gastrointestinal tract. However, because of the high proline content of gluten, the degradation is not efficient and relatively large gluten peptides can persist.29 There are thousands of such gluten peptides produced during the digestive process, and multiple peptides can stimulate an immune response in an individual. The protease-resistant 33-amino acid peptide from wheat α-gliadin is the immunodominant antigen in wheat, but little is known about the hierarchy of immunodominance and consistency of recognition of T-cell epitopes *in vivo*.30 Although the majority of immune reactions to gluten peptides is due to binding to HLA-DQ2, some gluten peptides have a different antigenic specificity from that of CD and are independent of the action of transglutaminase enzyme and HLA-DQ2/DQ831 and therefore are directly presented by antigen presenting cells to T cells. Further cooperation with B cells results in antibody production to the antigens.

*Figure 2 – The kernel of wheat is comprised of hundreds of proteins. These molecules can be classified as either water- or alcohol-soluble. Proteins and peptides from each category can potentially be pathogenic to the Gluten-Reactive patient. By assessing both water- and alcohol-soluble fractions of wheat, a clearer picture of Gluten-Reactivity can be obtained.*
Up to 86% of patients recognize a different array of peptides. And yet, commercially, the only peptide that is tested is alpha-gliadin 33 MER. A panel of gluten peptides, which includes a number of the more common immunodominant antigens, would provide new opportunities to screen, prevent disease development in individuals at risk, and increase the sensitivity of the test to identify Gluten-Reactivity (with or without the enteropathy CD).

Antibody Array 3 includes testing for antibodies to the gluten peptides: native + deamidated \(\alpha\)-Gliadin-33-mer, \(\alpha\)-Gliadin-17-mer, \(\gamma\)-Gliadin-15-mer, \(\omega\)-Gliadin-17-mer and Glutenin-21-mer.

**OPIOID PEPTIDES**

Exorphins are peptides which may have activity similar to that of morphine and other opioids. Five distinct exorphins have been identified in the pepsin-digest of gluten. The inhibitory action of the exorphins in wheat has a specific opiate effect. This morphine-like psychoactive nature of the peptides results from the incomplete digestion of these dietary proteins binding to the opiate receptors in the brain, and offers a possible explanation for some of the reported psychiatric reactions to these gluten proteins, including the sense of ‘brain fog,’ behavioral problems, or mood swings that often accompany immune reactions to these foods and which may follow with panic attacks, depression, or other neurological complaints.

Antibody Array 3 includes testing for antibodies to the gluten opioid peptides: Gluteomorphin + Prodynorphin.

**LECTINS**

Wheat germ agglutinins (WGAs) are lectins or carbohydrate-binding proteins with a capacity to bind to many cells and tissue antigens. Lectins bind to cells involved in the immune system and induce toxic damage, inflammation, and autoimmunity. Most lectins, including WGAs, are resistant to proteolysis, the degradation of proteins by cellular enzymes. WGAs profoundly interfere with enzyme function and inhibit their digestive function. By the binding of lectins to different tissue antigens, WGA can enhance antibody production against itself as well as against the tissue and cells to which it binds. For example, in humans, WGA binds to the same surface receptors to which islet autoantibodies bind. Therefore, an islet cell with bound lectins would be a sitting duck for autoimmune diseases. In humans, the evidence incriminating wheat lectin as a cause of IgA nephropathy (IgAN) is now impressive. Children with IgAN have high blood levels of anti-gliadin and anti-mesangium antibodies, and their IgA is unusually lectin-sensitive. Also, there is circulating WGA in the blood of children with active IgAN, which may suggest that it is, in fact, wheat lectin from the diet that may be an initiator of the autoimmune response. A gluten-free diet in IgAN patients was shown to reduce proteinuria, IgA-immune complexes and IgA food antibodies.

Antibody Array 3 includes testing for antibodies to the wheat lectin: Wheat Germ Agglutinin.
ENZYMES

Enzymes complete this panel. Transglutaminases are a family of enzymes that form protein polymers, like scaffolding, which are vital in the formation of barriers and stable structures. Since humans have transglutaminase (tTG) in many other tissues, including bone, antibodies produced against epithelial cell tTG2 can cross-react with other tTGs such as bone, brain and skin. In such cases, this cross-reaction leads to autoimmune responses against other tissues and thus develops into osteoporosis, neuroautoimmunity and skin disorders. Generally, patients with elevated antibodies to tTG are susceptible to autoimmunity. tTG2 has been shown to form complexes with gliadin. The incubation of tTG2 with gliadin results in the formation of covalent tTG-peptide complexes, which can adhere to intestinal walls. This positioning allows the gliadin-tTG complex to be recognized by antigen-presenting cells, which produces an immune response cascade that results in autoantibodies. The production of these autoantibodies may perpetuate a pro-inflammatory gastrointestinal destructive cycle.

Tissue Transglutaminase-3 (tTG3) is expressed mainly in the epidermis, and to a lesser extent in the placenta and the brain. tTG3 has been shown to be up-regulated in a variety of degenerative diseases. In certain patients, gluten-sensitive enteropathy manifests as a disorder of the skin called dermatitis herpetiformis (DH). DH is characterized by granular IgA deposits in the papillary dermis, which contribute to polymorphic papules and blisters often located over extensor surfaces of the major joints. Patients with Huntington’s disease have been shown to make elevated antibody levels to Transglutaminase-2 and -3. Transglutaminase is activated by oxidative stress, during which inflammatory cytokine production increases, specifically tumor necrosis factor-alpha and interferon-gamma. Huntington patients have been shown to produce more interferon-gamma and interleukin-2 than healthy controls. Elevated tTG3 expression is in esophageal cancer.

Tissue Transglutaminase-6 (tTG6) is expressed in neural tissue. The tTG6 enzyme is not commonly expressed in the small intestine but can be found in mucosal antigen-presenting cells. Due to its close homology to tTG2 and tTG3, it provides a clear possibility that tTG6 could be involved in the pathogenesis of gluten reactivity–related neurological dysfunction. Researchers speculate that autoimmunity against tTG6 may result from early brain damage and associated inflammation. Patients with high levels of antibodies against tTG6 are suspected of having autoimmunity against neuronal tissue. Neuronal clinical conditions may manifest as Cerebral Palsy, Gluten Ataxia or Peripheral Neuropathy. Antibodies may appear in serum before the clinical onset of symptoms.

Antibody Array 3 includes testing for antibodies to enzymes: Tissue Transglutaminases -2, -3 and -6 and Gliadin-Transglutaminase Complex.
Measurements of antibodies against various wheat protein peptides and enzymes associated with autoimmunities are shown in Figure 3.

**Figure 3** – Antibody Array 3 measures IgG and IgA antibodies against wheat proteins, peptides and associated enzymes.

**INFLUENCING FACTORS:**

**GENETIC**
Of the general population, 40-50% are carriers of DQ2/DQ8 genes, however close to 90% of Celiac disease patients carry the gene DQ2 (DQA1*05/DQB1*02), and a minority (10%) of the Celiac disease patients carry DQ8 (DQA1*03/DQB1*0302). Typically, gluten peptides bind to the DQ2 and DQ8 molecules. Recent research however, has identified at least eight new genomic regions with robust levels of disease association to Gluten-Reactivity.57 58

**ENVIRONMENTAL (CHEMICALS, FOODS, BIOTOXINS, DRUGS…)?**
Environmental factors that have an important role in the development of CD have been suggested by epidemiologic studies. These include a protective effect of breast-feeding59 and the introduction of gluten in relation to weaning.60 61

Numerous environmental factors have been hypothesized as being catalysts for the development of not only the gluten enteropathy CD,62 but also systemic manifestations of Gluten-Reactivity with or without the enteropathy. Some of these catalysts include bacteria,63 viruses,64 dysbiosis,65 and cross-reactive foods.66

**HISTORY (FAMILY, MEDICAL)**
CD and Gluten-Reactivity are characterized by a variety of clinical manifestations. These include the typical malabsorption syndrome (classic symptoms) and a spectrum of symptoms potentially affecting any organ or body system (non-classic symptoms).6 67 68

Clinical manifestations of Gluten-Reactivity and CD can present at any age:
Infancy (less than 2 years old) – diarrhea, abdominal distention, failure to thrive (low weight, lack of fat, hair thinning), anorexia, vomiting, psychomotor impairment (muscle wasting)

Childhood – diarrhea, constipation, anemia, loss of appetite, short stature, osteoporosis

Adulthood – diarrhea, constipation, anemia, aphthous ulcers, sore tongue & mouth (mouth ulcers, glossitis, stomatitis), dyspepsia, abdominal pain, bloating (weight loss), fatigue, infertility, neuropsychiatric symptoms (anxiety, depression, etc.), bone pain (osteoporosis), weakness (myopathy, neuropathy).

Reviewing current medications (antibiotics, steroids, NSAID’s, etc.), supplements, diets, and a detailed medical history are critically important in determining who may have gluten sensitivity. The correlation between food ingestion and symptom onset is of great clinical importance.

**CLINICAL – SYSTEMIC IMMUNE EFFECTS**

Inclusion of the specific antigens comprising Antibody Array 3, or Comprehensive Gluten-Reactivity and Autoimmunity is based on recent medical research studies. Comprehensive quantitative mapping of T-cell epitopes was determined in CD. Results demonstrated that patients respond to a heterogeneous array of peptides; some recognized many peptides from single or multiple gliadin families, while others reacted to only one peptide. These results confirmed that a large number of gluten epitopes may be implicated in the development of CD and associated diseases. Indeed, a T-cell line from one Celiac patient failed to recognize any of the 21 tested peptides, which confirmed that a large number of gluten and other wheat protein epitopes are implicated in development of CD and associated disorders. This suggests that other gliadin peptides and proteins are involved in the pathogenesis of Gluten-Reactivity and CD. We extended this heterogeneity in T-cell responses to gluten and other peptides originated from wheat to humoral immune responses by measuring IgG and IgA antibodies against nine different wheat antigens and peptides as well as enzymes associated with autoimmunities. Heterogeneity in IgG and IgA antibodies against these twelve antigens was confirmed by variation in antibody response against various wheat associated antigens on individual bases. Therefore, Antibody Array-3, with its measurement of IgG and IgA antibodies against a repertoire of proteins, enzymes and peptides, originated not only from α-gliadin but also from γ- and Ω-gliadin, glutenins, agglutinins, opioid peptides (Gluteomorphin + Prodynorphin), and specific enzymes involved in the pathogenesis of CD and Gluten-Reactivity, enhances the clinical sensitivity and specificity for the detection of CD and Gluten-Reactivity.

**CLINICAL USE OF ANTIBODY ARRAY 3**

Measuring a patient’s immune response to an array of wheat antigens increases the sensitivity and specificity, and will provide greater confidence in formulation of a diagnosis that allows for better patient compliance with a gluten-free diet. Assessing wheat/gluten reactivity and intestinal autoimmunity is recommended for patients who:
• Have gut dysbiosis, which appears to be resistant to standard therapy
• Are suspected of having intestinal mucosal damage
• Complain of food allergy and intolerance
• Complain of chemical hypersensitivity
• Present multiple-symptom complaints (including Chronic Fatigue Syndrome and Fibromyalgia)
• Suffer from abnormal immune cell count and function
• May suffer from blood-brain barrier permeability, depression, or neuroautoimmunity
• Neuroautoimmune patients to consider:
  o Thyroiditis
  o Arthritis
  o Myocarditis
  o Dermatitis
  o Endocrinopathy
  o Polyendocrinopathy
  o Osteoarthritis
  o Pernicious Anemia
  o Other

**CLINICAL INTERPRETATION OF ANTIBODY ARRAY 3**

When IgA reactions are predominant, it is an indication of possible Celiac disease and other autoimmunities.

When IgG reactions are predominant, it is an indication of wheat/gluten immune response and possible autoimmunity due to lack of digestive enzymes and/or other factors.

When both IgA and IgG reactions occur, it is an indication of wheat/gluten immune response and its progression to Celiac disease and/or other autoimmune disorders.
# Antibody Array 3 – Wheat/Gluten Proteome Reactivity & Autoimmunity

## Interpretation of Antibodies Against Wheat, Gluten, Enzyme Antigens

<table>
<thead>
<tr>
<th>Positive Reaction To:</th>
<th>Gluten-Sensitivity</th>
<th>Wheat &amp; Gluten-Sensitivity</th>
<th>Wheat-Sensitivity</th>
<th>Lectin-Sensitivity</th>
<th>Autoimmune Reaction</th>
<th>Interpretation</th>
<th>Clinical Approach</th>
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</thead>
<tbody>
<tr>
<td>Wheat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Wheat sensitivity due to lack of digestive enzymes</td>
<td>Wheat-free diet  Heal gut</td>
</tr>
<tr>
<td>WGA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sensitivity to wheat germ and sprouted wheat</td>
<td>Check for other lectin sensitivity</td>
</tr>
<tr>
<td>γ-Gliadin-15-mer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>One or any combination of positives means sensitivity to specific gluten epitopes due to lack of enzymes, in particular DPPIV</td>
<td>Gluten-free diet  Heal gut  Check for extra-intestinal autoimmunity</td>
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<td>α-Gliadin-17-mer</td>
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<td>α-Gliadin-33-mer</td>
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<td>ω-Gliadin-17-mer</td>
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<td>Glutenin-21-mer</td>
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<tr>
<td>Gluteomorphpin + Prodynorphin</td>
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<td></td>
<td></td>
<td></td>
<td>Immune reaction to opioid peptides. Due to lack of digestive enzymes, in particular DPPIV</td>
<td>Patient is “addicted” to wheat</td>
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<tr>
<td>Gliadin-tTG2 Complex</td>
<td></td>
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<tr>
<td>tTG2</td>
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<td></td>
<td></td>
<td>Autoimmunity associated with wheat sensitivity</td>
<td>Remove trigger  Use anti-inflamatories</td>
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<tr>
<td>tTG3</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Possible skin autoimmunity associated with wheat sensitivity, if wheat antigens are also positive</td>
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<tr>
<td>tTG6</td>
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<td></td>
<td></td>
<td>Possible brain autoimmunity associated with wheat sensitivity, if wheat antigens are also positive</td>
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SPECIMEN REQUIREMENT

2 mL Serum
Ambient

RELATED TESTING

- Antibody Array 2 - Intestinal Antigenic Permeability Screen (Serum)
- Antibody Array 4 - Gluten-Associated Cross-Reactive Foods and Foods Sensitivity (Serum)
- Antibody Array 5 – Systemic Autoimmune Reactivity Screen (Serum)
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