ARRAY 2

ARRAY 2 – Antibody

Intestinal Antigenic Permeability Screen™

Cyrex™ Laboratories

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CLINICAL APPLICATION GUIDE TO INTESTINAL ANTIGENIC PERMEABILITY SCREEN™

OVERVIEW

At What Are We Looking?

Research confirms that the root cause for many of undesired immune reactions originates in the gastrointestinal tract. GI tract abnormality can compromise the integrity of the gut barrier and increases the entry of undigested antigens into the sub-mucosa and the circulation, thus challenging the immune system. Reaction to these antigens activates immune and inflammatory cascades, resulting in the production of pro-inflammatory cytokines and an array of antibodies, which further contributes to increased intestinal barrier permeability (or "leaky gut" syndrome).

INTESTINAL BARRIER DYSFUNCTION: MECHANISMS

Current Methodologies

The current methodology for assessing intestinal permeability uses lactulose and mannitol. Over the last 40 years, it has been a useful clinical tool. Lactulose absorption suggests a tear in the gut barrier, and thus, intestinal permeability. Against popular belief, the absorption of this small molecule actually indicates a minute leak rather than a tear. Lactulose has relatively low molecular size, and the transfer of this substance through the gut membranes does not reflect the situation for transfer of food protein and immune response. Furthermore, Lactulose/Mannitol test measures the transfer of small molecules only through paracellular but not transcellular pathway. Therefore, Large Molecule Intestinal Permeability Identification (LMIPI) should be assessed using large molecules comparable to the size of food proteins, which are antigenic and challenge the immune system. See Figure 1 for the triggers and biomarkers of abnormal intestinal permeability and how to use the next generation of testing for Intestinal Permeability Identification.

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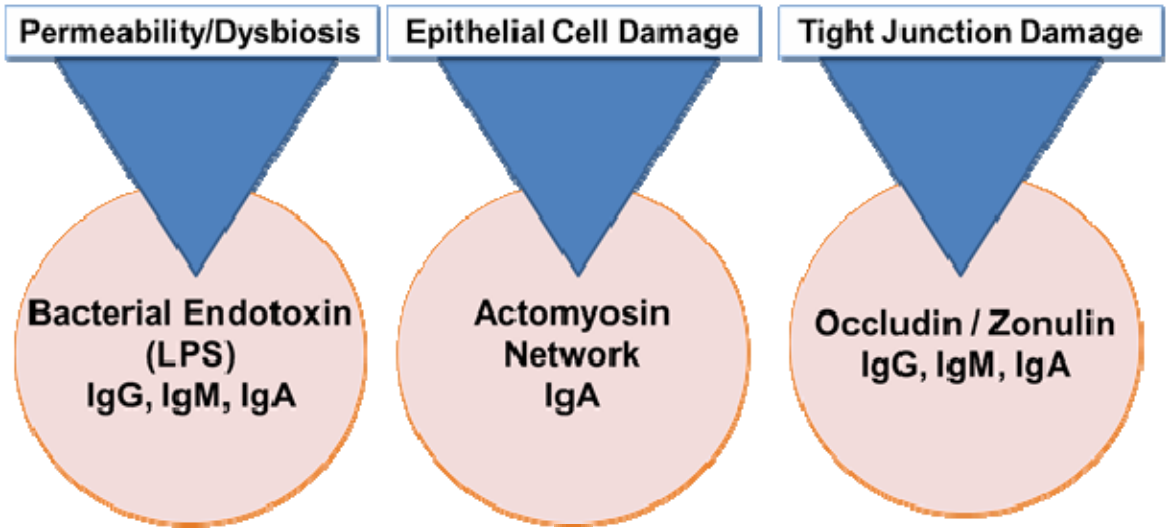

Figure 1 – *Biomarkers of Intestinal Permeability Identification*

We have been warned for over 2000 years that the health of the body is heavily influenced by the gut. Hippocrates told us, "bad digestion is the root of all evil." [1] The idea was further promoted in the 19th Century by the great naturopath Louis Kühne and in the early 20th Century by Nobel laureate Elie Metchnikoff. Kühne proposed that an inappropriate diet led to intestinal toxicity, with increased growth of bacteria in the bowel causing disease. Elie Metchnikoff won the Nobel Prize in Medicine for his work on the milieu of the intestines. One of his most famous messages to us? "Death begins in the colon." [2] A century later we are beginning to understand some of the physiological mechanisms that underlie the observations of this pioneer.

The exaggerated entrance of antigenic macromolecules across the gut epithelium can initiate production of, and perpetuate an ongoing increase in, multiple inflammatory cytokines [3] and systemic chronic inflammation. This appears to be a required component for the trio of factors that lead to eventual autoimmune disease (genetic vulnerability, environmental exposure, and intestinal permeability).[4]

The term epithelium refers to cells that line hollow organs and glands and those that make up the outer surface of the body, which protect or enclose organs. Most produce mucus or other secretions.[5] 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 single-cell epithelial lining in the intestines forms an amazingly large surface area (300-m^2, about the size of a tennis court), which represents the single largest interface between our bodies and the external environment.[6] The function of intestinal mucosa is far more than just transportation of nutrients; it balances the needs for a barrier against a hostile environment, like the skin, with the necessity of active and passive transport, like the renal tubule. The intestinal mucosal cells favor fluxes of nutrients, regulate ion and water movements, and limit host contact with the massive intraluminal load of dietary antigens and microbes.[7] An intact intestinal barrier is, therefore, critical to normal physiological function and the prevention of disease.[8]

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A human will have consumed between three and seven tons of food in a lifetime. Much of this food carries potentially antigenic material (bacteria, molds, fungus, yeast, viruses, etc.). Some of these antigens pose no threat to the mucosal immune system, while others may be harmful to the host. Although strong protective immunity is essential to prevent invasion by pathogens, equivalent responses of the immune system against dietary proteins or commensal bacteria can lead to chronic disease.[9] For example, in genetically pre-disposed individuals, a single-dose of gluten may cause increased intestinal permeability.[10] Excessive exposure of these antigens to the intestinal immune system may cause the breakdown of the intestinal barrier, from a slight leakage to outright breaks, thus allowing for the entry of excessive amounts of larger molecules (macromolecules) into the body. When a breakdown in normal gut homeostasis occurs, the consequences can be devastating.[11] As the amount of antigenic molecules increases, the barrier integrity becomes overloaded, compromised, and a larger number of macromolecules is absorbed into the body.[12]

Oral tolerance generally refers to the suppression of immune responses following exposure of potential antigens through the oral route. Although the involvement of lymphoid cells, intestinal mucosa and the role of secretory IgA was recognized in the 1970s, our understanding of mechanisms involved at molecular levels has been tremendously increased over the past few decades. The role of regulatory T-cells, populations of cytokines such as Transforming Growth Factor-beta-1, Interleukin (IL)-10, and IL-4, modulation of immunoglobulin production especially secretory IgA, the type and size of the antigens, and the type of antigen presentation have been explained over the past few years.[13] [14] [15] [16] [17] [18] [19] [20] [21]

Permeability to a minor amount of antigenic molecules is considered normal in the human digestive tract (Figure 2).[22] Generally, the larger the molecule, the less likely it is to be allowed access to systemic circulation.[23] The intestinal immune system monitors these antigens in the lumen where they can interact with the mucosal and systemic immune system,[24] thus developing oral tolerance.[25] Unresponsiveness or tolerance to these antigens is maintained by three principal mechanisms: anergy or functional unresponsiveness, deletion through programmed cell death or apoptosis, and immune suppression by regulatory T-cells (Tregs). The mechanism of immune suppression or anergy to gliadin is shown in Figure 2. However, if the intestinal immune system is dysregulated, the oral tolerance can break down first, which may be followed by enhanced intestinal permeability. Therefore, defective oral tolerance can lead to gut inflammatory disorder, food allergy, Celiac disease and other autoimmune conditions.[26]

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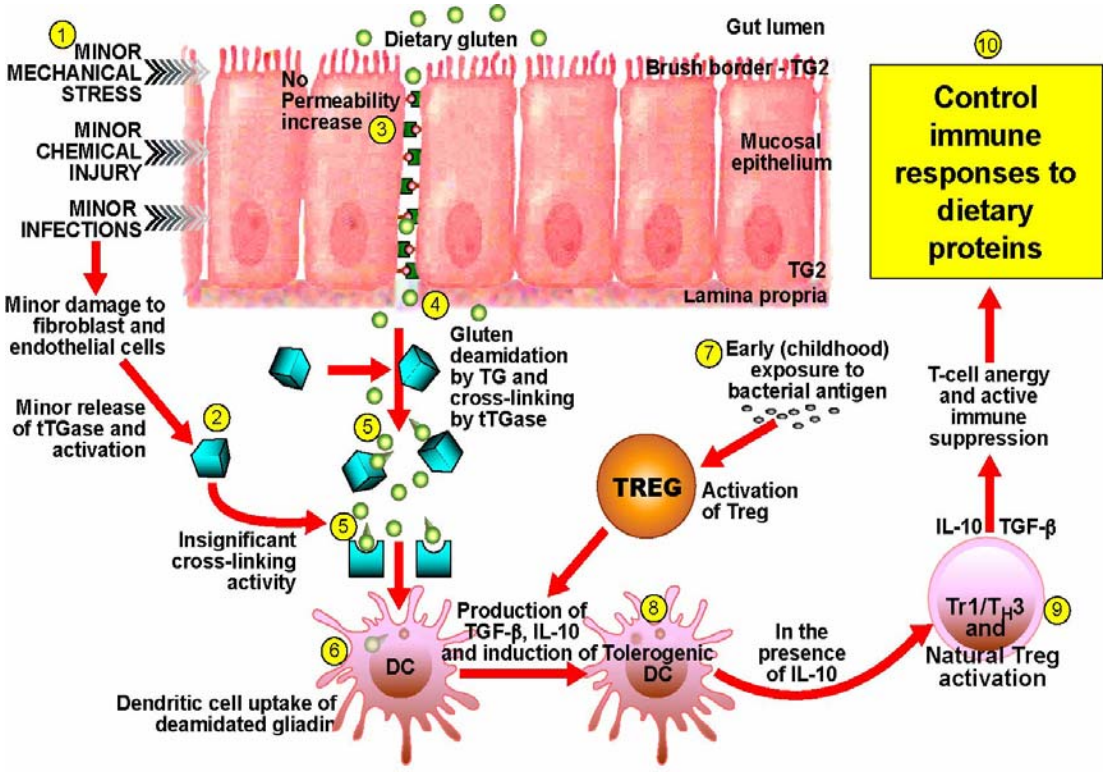

Figure 2 *- The Role of Regulatory T-Cells (7) in the Induction of Oral Tolerance to Dietary Proteins. Normal function of intercellular junction may allow the passage of few molecules of dietary gluten (3). These molecules are deamidated by enzyme tissue transglutaminase (4, 5), released from local cells due to stressor factors (1). Dendritic cells are responsible for processing and presenting antigens to immune T-cells and triggering immune reaction. (6) Regulatory cells can orchestrate the immune toward tolerance rather than reaction by promoting TGF-beta and IL-10. (8,9,10)*

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CLINICAL SIGNIFICANCE

Increased permeability of the intestinal barrier to macromolecules is associated with a variety of local and systemic inflammatory conditions. Intestinal permeability can cause systemic inflammation,[27] which can then sustain itself by its ongoing effect on the gut.[28] The target tissue damage is primarily determined by genetics and exposure of environmental factors,[29] leading to various clinical conditions including:

- Gluten Sensitivity and Celiac disease
- Food Allergies
- Inflammatory bowel disease (Ulcerative Colitis and Crohn's disease)
- Numerous autoimmune diseases (Rheumatoid Arthritis, Psoriasis, Type 1 Diabetes, Spondylitis, etc.)
- Neurological conditions (Multiple Sclerosis, Guillain Barré Syndrome, etc.)
- Cognitive Dysfunction (depression, anxiety, Schizophrenia, etc.)
- Others

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PATHOPHYSIOLOGY (MECHANISMS OF TISSUE DAMAGE)

Genetic predisposition, miscommunication between innate and adaptive immunity, exposure to environmental triggers, and loss of intestinal barrier function secondary to dysfunction of intercellular tight junctions all seem to be key components in the pathogenesis of autoimmune diseases.[30]

Gastrointestinal and autoimmune disorders are accompanied by an increased translocation of endotoxins and other bacterial toxins from aerobic and anaerobic bacteria through the gut wall. This increased translocation and the inflammation associated with it may induce degradation of tight junction proteins and a subsequent immune response against tight junction proteins such as occludin/zonulin and bacterial endotoxins such as lipopolysaccharides (LPS). Indeed, many chronic conditions are accompanied by increased serum levels of IgA and IgM against LPS and other antigens of pathogenic bacteria. Thus, the increased serum IgA and IgM against LPS and tight junction proteins (occludin/zonulin) indicate the presence of intestinal barrier permeability and trafficking of macromolecules through the tight junction, which may result in inflammatory and autoimmune conditions.[31] [32] [33] [34] [35]

Gastrointestinal microfilaments of the Actomyosin Network are critical for apical junctional complex biogenesis and function. The apical junctional complex, made up in part by tight junction proteins zonulin and occludin, is responsible for preventing antigen invasion and preservation of the biochemical homeostasis within the gastrointestinal tract. The Actomyosin Network can signal tight junction contractions and give structure to their assembly.

Many conditions, including gut inflammation, can cause the mucosal barrier to become more permeable, whereby enlarged spaces between the cells of the gut wall and dissociation of tight junction proteins can induce a loss of protective barrier. This compromised barrier may increase bacterial translocation, and thus, enhance the concentration of serum endotoxins and tight junction proteins. The endotoxins of bacteria may initiate an autoimmune response through bacterial toxins acting as superantigen to T lymphocytes, or by a mechanism called molecular mimicry. Many bacteria have antigenic sites very similar to human tissue antigens, including neuronal tissue. These antigens and the antibodies produced against them will go in turn into various tissues and trigger first inflammation and then autoimmunity.

Thus, patients with chronic inflammatory and autoimmune conditions should be checked for the existence of increased gut permeability by measurement of IgA and IgM against bacterial LPS and tight junction proteins, and IgG against actomyosin. Therefore, in many cases increased intestinal permeability precedes disease and causes an abnormality in antigen delivery that triggers the multi-organ process leading to autoimmune condition.[36] [37] [38] The exaggerated entrance of antigenic macromolecules across the gut epithelium might initiate production of, and perpetuate an ongoing increase in, multiple inflammatory cytokines[39] and systemic chronic inflammation.[40]

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INFLUENCING FACTORS:

GENETIC

Genetic vulnerability has been suggested in many studies.[41] For example, up to 70% of asymptomatic first-degree relatives of Celiac disease patients are positive for intestinal permeability.[42]

ENVIRONMENTAL

There have been many studies showing a variety of environmental factors that can affect mucosal permeability and initiate immunological inflammatory cascades. These include:

- Dysbiosis[43] and microorganism invasions of (bacteria,[44] yeast,[45] viruses,[46] parasites[47])
- Traumas including surgical and non-surgical lesions
- Stress[48] due to disease, starvation, sustained strenuous exercise,[49] radiation[50 51]
- Other environmental factors such as medications,[52] allergenic foods[53 54]

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FAMILY HISTORY

Reviewing current medications, supplements, and diets, and a medical history may be critically important in determining who may have intestinal permeability. Consider:

- Current or past use of antibiotics,[55] steroids,[56] and NSAIDS[57]
- History of chemotherapy or radiation treatments[58]
- History of chronic yeast infections[59]
- Digestive enzyme insufficiencies
- Diet history
    - Standard American Diet
    - low fiber diets
    - excessive alcohol or caffeine consumption
    - unknown exposure to food allergens used as fillers in cosmetics, medications, nutrients

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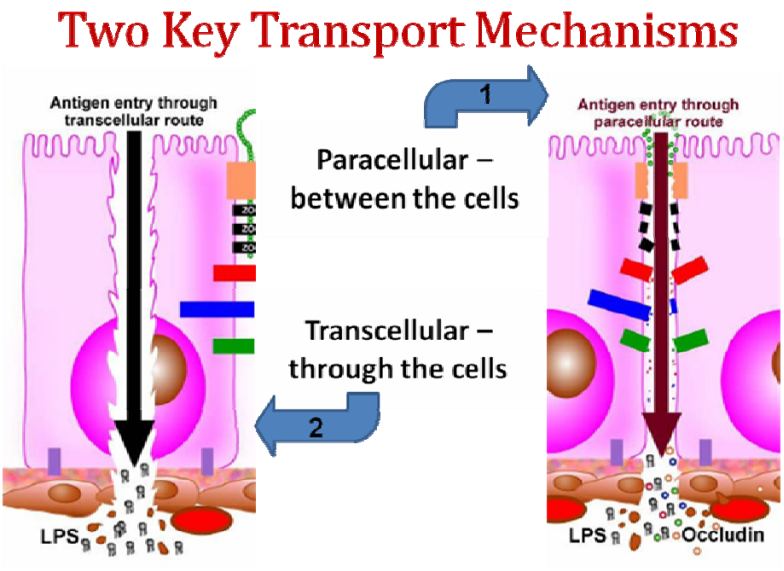

Figure 3 - *Macroscopic arrangement and microscopic composition of intercellular tight junctions, which consist of integral membrane proteins (occludin, claudin, and zonulin). The picture on the left represents the entry of antigen through the body of a cell (transcellular route). On the right it is shown how the antigens are transported between the cells (paracellular route).*

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MEASURING INTESTINAL PERMEABILITY

Two decades ago, the assessment of Intestinal Permeability was done by measuring the leakage of a sugar molecule (lactalose) into the blood stream. Since then, numerous studies have questioned the clinical relevance of this marker. It is not measuring antigenic macromolecule passage,[60] [61] [62] because passage of very small molecules such as sugars, lacks a capacity to challenge the immune system. Therefore, measurements of intestinal permeability to antigenic molecules are assessed not only against the triggers of intestinal barrier degradation such as bacterial endotoxins, but also against barrier structures occludin /zonulin and actomyosin, which represents both paracellular and transcellular pathways (see Figure 3).

'Therefore, macromolecular intestinal permeability, which stimulates an immune response, can be accurately measured by identifying either antibodies to antigenic molecules, which trigger intestinal barrier degradation such as bacterial endotoxins, or antibodies against the components involved in the intestinal barrier degradation such as occludin/zonulin and actomyosin.'

Elevated antibodies to LPS, Occludin/Zonulin and the Actomyosin Network are patent pending biomarkers that identify the breakdown of a healthy intestinal barrier, which allow penetration of large antigenic molecules: [63] [64] [65]

1. Lipopolysaccharides (LPS) are large molecules found in gram-negative bacteria. They are endotoxins, and if absorbed, elicit a strong immune response.[66] The detection of antibodies against LPS reveals macromolecule-sized endotoxin infiltration through the intestinal barrier into the systemic circulation.

2. Occludin is part of the main component of proteins holding together the tight junctions. The detection of antibodies to occludin indicates that the tight junctions are breaking down. This is a measure of a mechanism involved in damaging the intestinal barrier membrane.

3. Zonulin, a protein, regulates the permeability of the intestine.[67] The detection of antibodies against zonulin indicates that the normal regulation of tight junctions is compromised. This can be a clue to presence of an ongoing mechanism involved in damaging the intestinal barrier.

4. The Actomyosin Network, a protein complex, regulates intestinal barrier function by maintaining the plasticity of tight junctions.[68] Antibodies to the actomyosin network are a biomarker of intestinal barrier dysregulation via cell infiltration. For example, 98.2% of Celiac disease patients with flat mucosa have antibodies to actin.[69] This is a measure of mechanism involved in damaging the intestinal barrier.

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Antibody Array 2 for assessment of intestinal barrier integrity measures antibodies against bacterial endotoxins (lipopolysaccharides), tight-junction proteins (occludin, zonulin) and cell cytoskeleton (actomyosin), and identifies both transcellular and paracellular routes of intestinal barrier penetration by large molecules with a capacity to challenge the immune system.

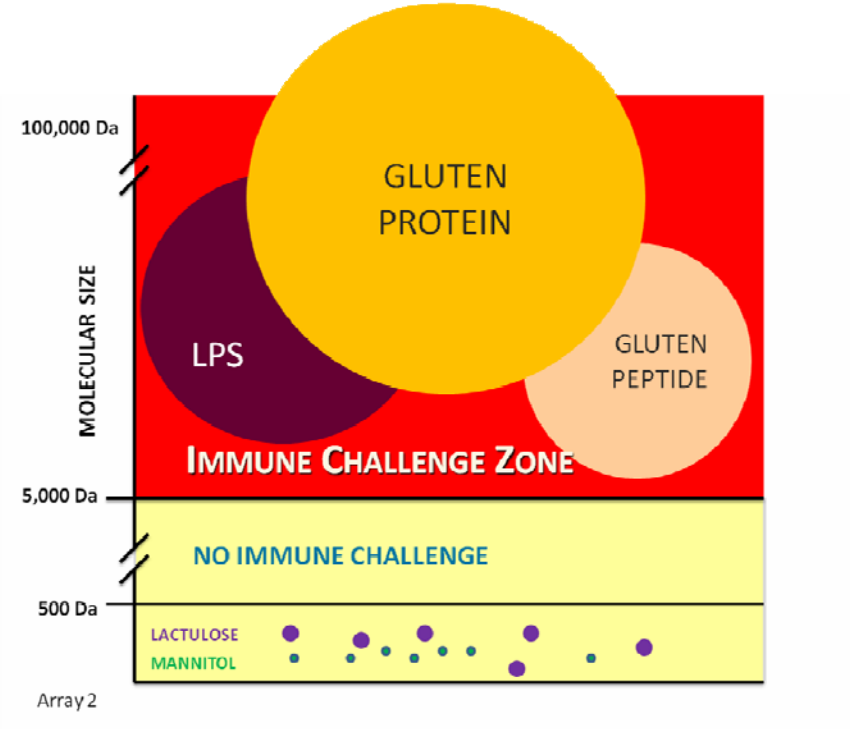

Figure 1 *- Intestinal Permeability Identification (IPI) requires the assessment of large molecules, which elicit an immune response when infiltrating the submucosa.*

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CLINICAL USE OF ANTIBODY ARRAY 2

The development of autoimmunity requires three ingredients: genetic predisposition, an environmental trigger and increased intestinal permeability. Intestinal barrier integrity plays a vital role in the overall health and well-being of patients. Those with a family history of autoimmunity or neurodegeneration should be assessed regularly. In addition, patients who present with multiple symptom complaints or complain of food allergy or intolerance may have increased intestinal barrier permeability. Measurement of intestinal barrier permeability 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
- Present multiple symptom complaints (including Chronic Fatigue Syndrome)
- Are suspected of suffering from blood-brain barrier permeability, depression, or neuroautoimmunity

CLINICAL INTERPRETATION FOR ANTIBODY ARRAY 2

Interpretation of elevated level of antibodies against LPS, occludin/zonulin and actomyosin is shown in Table 1.

Interpretation of Antibodies Against LPS, Occludin / Zonulin and Actomyosin Network					
LPS IgA, IgM or IgG	+	+	-	+	-
Occludin/ Zonulin IgA, IgM or IgG	-	+	+	-	-
Actomyosin IgA	-	-	-	+	+
Clinical Indication	Gut flora dysbiosis	Breakdown in intestinal barrier integrity by bacterial antigens through paracellular pathway	Breakdown in intestinal barrier integrity by factors other than bacterial antigens, through paracellular pathway	Breakdown in intestinal barrier integrity by bacterial antigens through transcellular pathway	Autoimmunity against mucosal epithelium and other tissue cell cytoskeleton including Celiac disease, Chronic Active Hepatitis and primary biliary cirrhosis
Clinical Approach	Pre- & Pro-biotics	Pre- & Pro-biotics Heal the gut	Reduce stress Heal the gut	Pre- & Pro-biotics	Anti-inflammatories

Table 1- elevated level of antibodies against LPS, Occludin/Zonulin and Actomyosin and related clinical correlation

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Intestinal permeability is significant in gastrointestinal autoimmune disease.[70] The following diagrams compare the elevation of antibodies against bacterial endotoxins (Lipopolysaccharides) and the structure of tight junctions (occludin/zonulin) in healthy controls and patients with gastric autoimmunity done at Cyrex Labs.

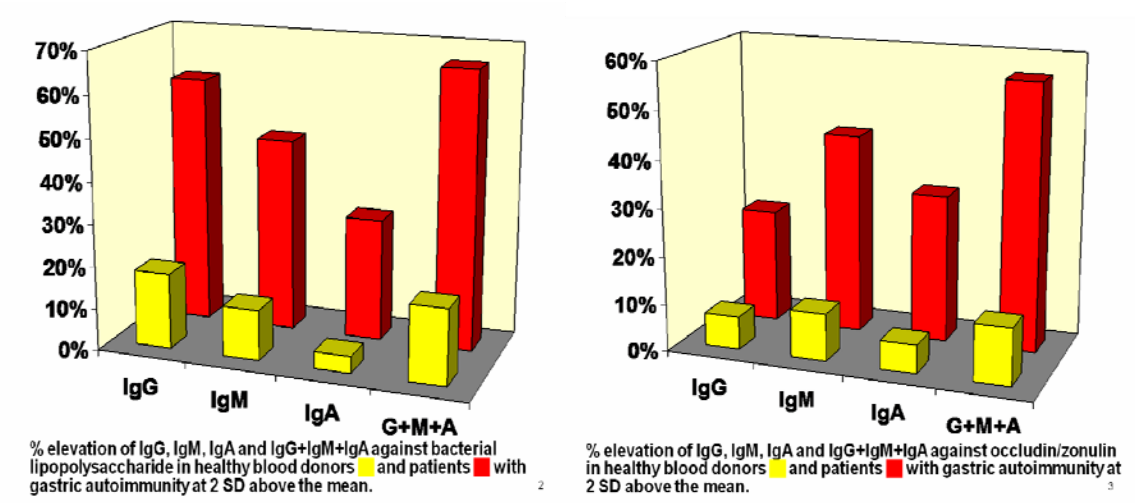

Figure 5 – *A comparison of IgG, IgM and IgA against bacterial lipopolysaccharide and occludin/zonulin in healthy donors and patients with gastric autoimmunity.*

The exaggerated entrance of antigenic macromolecules across the gut epithelium can initiate production of, and perpetuate an ongoing increase in, multiple inflammatory cytokines[71] and systemic chronic inflammation.[36] This appears to be a required component for the trio of factors that lead to eventual autoimmune disease (genetic vulnerability, environmental exposure, and intestinal permeability).

The Antibody Array Panel 2 – Intestinal Antigenic Permeability Screen is an extremely sensitive marker of the dysfunctional epithelial barrier, allowing macromolecular penetration from the lumen of the intestines into systemic circulation.

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SPECIMEN REQUIREMENT

2 mL serum
Ambient

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RELATED TESTING

- **Antibody Array 1 - Mucosal Gluten Reactivity Screen (Oral Fluid)**
- **Antibody Array 3 - Wheat/Gluten Proteome Reactivity and Autoimmunity (Serum)**
- **Antibody Array 4 - Gluten-Associated Cross-Reactive Foods and Foods Sensitivity (Serum)**
- **Antibody Array 5 - Neuroautoimmunity Panel (Serum)**

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