

# **ASSESSMENT OF INTESTINAL BARRIER FUNCTION TEST USING SALIVA**

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## INTRODUCTION:

The intestinal epithelium is the largest mucosal surface in the human body that provides an interface between the external environment and the host. In the gut, two key elements govern the interplay between environmental triggers and the host:

- 1. Intestinal permeability**
- 2. Intestinal mucosal defense**

The permeability of the intestinal epithelium depends on the regulation of the mucosal immune system and intercellular tight junctions. Research carried out in the last decade has demonstrated that tight junctions are composed of a complex meshwork of proteins, the interaction of which dictates their competency.

Zonulins, occludin, claudins and junctional adhesion molecules are a few examples that modulate movement of fluid, macromolecules and leukocytes from the blood stream to the intestinal lumen, and vice versa. In addition, these tight junction proteins are involved in protecting the epithelial cells of the intestine against colonization by microorganisms. It is now apparent that tight junctions are dynamic structures that are involved in developmental, physiological and pathological processes. As a result, particular attention is being placed on the role of tight junction dysfunction in the pathogenesis of several diseases, particularly autoimmune diseases.

Pathophysiological regulation of tight junctions is influenced by many factors, including: secretory IgA, enzyme, neuropeptides, neurotransmitters, dietary peptides and lectins, yeast, aerobic bacteria and anaerobic bacteria, parasites, proinflammatory cytokines, free radicals and regulatory T-cell dysfunction.

Given the complexity of intracellular structure and function of tight junction proteins, it is not surprising that when tight junction proteins are affected, the physiological state of epithelial and/or endothelial cells is dramatically changed as well.

In fact, tight junction dysfunction seems to be the primary defect in autoimmune diseases.

## **Intestinal Mucosal Defense**

Paracellular passage of macromolecules under either physiological or pathological circumstances is safeguarded by gut-associated lymphoid tissue (GALT). GALT serves as a containment system that prevents potentially harmful intestinal antigens from

reaching the systemic circulation, and induces systemic tolerance against luminal antigens by a process that involves polymeric IgA secretion and induction of T-regulatory-cell activity and immune tolerance.

The balance between immunity and tolerance is essential for a healthy intestine; abnormal or inappropriate immune responses can result in inflammatory pathologies.

### **The Intestinal Neuroendocrine Network**

Intestinal homeostasis is coordinated by the responses of different cell types, including both immune and nonimmune cells. The interaction between immune and nonimmune cells is amplified by the influx of inflammatory/immune cells, which increases the exposure of nonimmune cells to soluble mediators (e.g., cytokines) released from immune cells. Macrophages, leukocytes and mucosal mast cells (MMC) all release several mediators that alter gut function. Of interest is that MMCs seem to have a role in both T<sub>H</sub>1-driven and T<sub>H</sub>2-driven responses. MMCs release several preformed mediators, such as histamine, serotonin and mast-cell proteases, as well as newly synthesized mediators including leukotrienes, prostaglandins, and platelet-activating factor, in addition to interleukin-4 and TNF- $\alpha$ ; many of these mediators affect epithelial permeability. This might explain, in part, the increased intestinal permeability that is a feature of both T<sub>H</sub>1-mediated and T<sub>H</sub>2-mediated pathologies.

### **Increased Intestinal Permeability and a Paradigm Shift in the Pathogenesis of Autoimmune Diseases**

A common denominator in autoimmune diseases is the presence of several pre-existing conditions that lead to an autoimmune process. The first of these conditions is the genetic susceptibility of the host immune system to recognize, and potentially interpret, an environmental antigen presented within the gastrointestinal tract. The second is that the host must be exposed to the antigen. Finally, the antigen must be presented to the gastrointestinal mucosal immune system following its paracellular passage from the intestinal lumen to the gut submucosa; this process is normally prevented by competent tight junctions. In many cases, increased intestinal permeability seems to precede disease and causes an abnormality in antigen delivery that triggers the multiorgan process leading to the autoimmune response.

The pathogenesis of autoimmune diseases can therefore now be described by three key points. First, autoimmune diseases involve a miscommunication between innate and adaptive immunity. Second, molecular mimicry or bystander effects alone might not explain entirely the complex events involved in the pathogenesis of autoimmune diseases. Third, in addition to genetic predisposition and exposure to triggering nonself-antigens, the loss of protective function of mucosal barriers that interact with the environment (mainly the gastrointestinal and lung mucosa) is necessary for autoimmunity to develop.

### **Food Hypersensitivity and Clinical Outcome of Impaired Intestinal Permeability**

Food sensitivity is the best testament to the accuracy of the new paradigm for the pathogenesis of autoimmunity proposed above. This intestinal disorder is a unique model of autoimmunity.

Early in the development of food sensitivity, tight junctions are opened, most likely secondary to zonulin upregulation, and severe intestinal damage ensues. The upregulation of the zonulin innate immunity pathway is directly induced by exposure to the disease's antigenic trigger. Dietary peptides have also been shown to be a potent stimulus for macrophage proinflammatory gene expression and for cytokine release. Data indicate that dietary peptides initiate intestinal permeability through a MyD88-dependent release of zonulin that enables paracellular translocation of peptides and their subsequent interaction with macrophages within the intestinal submucosa.

The interaction of peptides with macrophages initiates signaling through a TLR-like pathway, which results in the establishment of a proinflammatory (T<sub>H</sub>1-type) cytokine milieu and subsequently mononuclear cell infiltration into the submucosa. This, in turn, might permit the interaction of T cells with antigen-presenting cells, including macrophages, ultimately leading to the antigen-specific adaptive immune response seen in patients with food sensitivity. Once peptides are removed from the diet, serum zonulin levels decrease, the intestine resumes its baseline barrier function, autoantibody titers are normalized, the autoimmune process turns off and, consequently, the intestinal damage (which represents the biological outcome of the autoimmune process) heals completely.

In fact, in clinically asymptomatic Crohn's disease patients, increased intestinal epithelial permeability precedes clinical relapse by as much as 1 year, indicating a permeability defect might be an early event in disease exacerbation.

Although a primary defect in intestinal barrier function might be involved in the early steps of the pathogenesis of IBD, the production of cytokines, including IFN- $\gamma$  and TNF- $\alpha$ , secondary to the inflammatory process, perpetuates the increased intestinal permeability.

Immunohistochemical localization of tight junction proteins in mucosal biopsies from IBD patients shows altered expression of several critical tight junction proteins, including upregulation of claudin 2, which might be due to the disruptive effects of proinflammatory cytokines on the barrier associated with internalization of these transmembrane proteins. On this manner, a vicious circle is created, in which barrier dysfunction allows further leakage of luminal contents, thereby triggering an immune response that in turn promotes further leakiness.

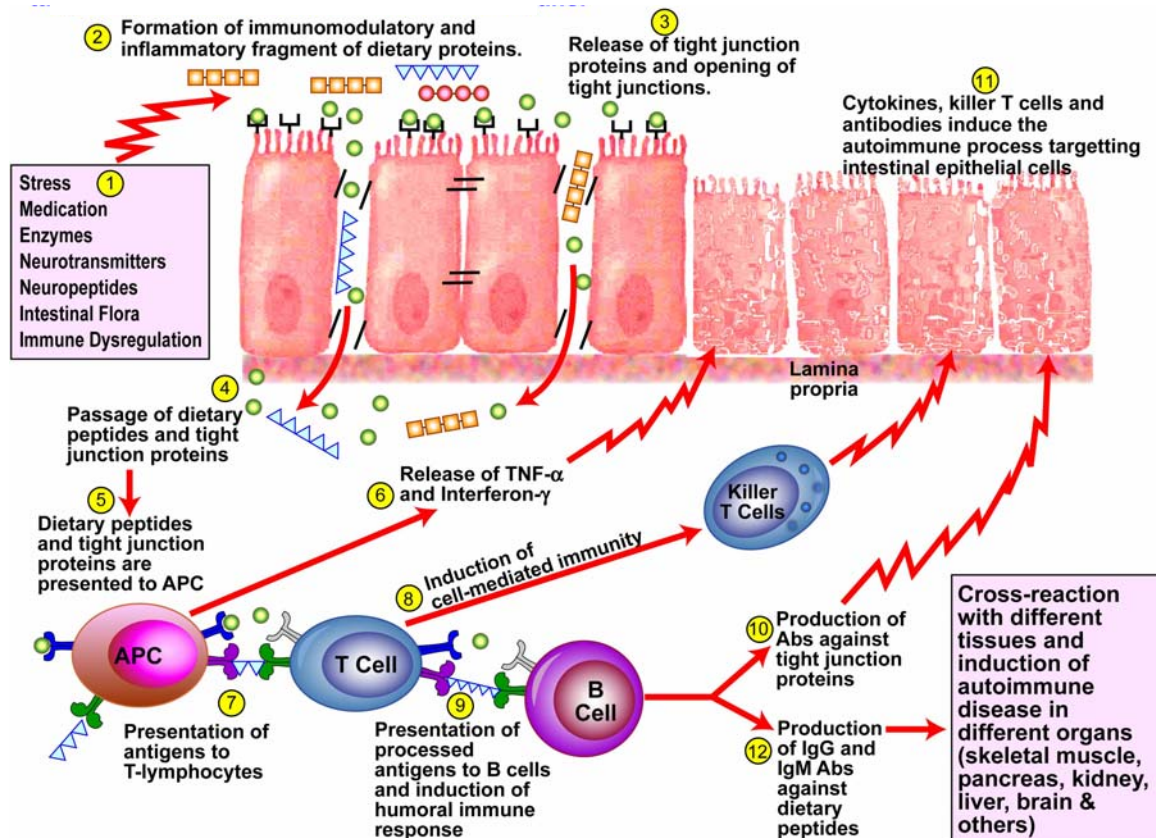
This 'breach' of the intestinal barrier by nonself-antigens might lead to an immune response targeting extraintestinal organs. These organs include, among others, the skeletal system (ankylosing spondylitis), the pancreas (type 1 diabetes), the kidney (IgA nephropathy), the liver (nonalcoholic steatohepatitis), and the brain (multiple sclerosis).

## **SUMMARY**

The classical paradigm of autoimmune pathogenesis involving a specific genetic makeup and exposure to environmental triggers has been challenged by the addition of a third element: the loss of intestinal barrier function. This new theory implies that, once the autoimmune process is activated, it is not self-perpetuating; rather, it can be modulated or even reversed by preventing the continuous interplay between genes and environment. As tight junction dysfunction allows this interaction, new therapeutic strategies aimed at re-

establishing the intestinal barrier function offer innovative, unexplored approaches for the treatment of these devastating autoimmune diseases that target many tissues and organs of the human system.

**Figure 1 – Proposed role of abnormal intestinal permeability in the pathogenesis of autoimmune disease targeting intestinal tissue and different organs.**



### Intestinal Barrier Function Test Using Saliva

The dominant antibody isotype of the mucosal immune system is IgA. In the blood IgA is found as a monomer, but in mucosal secretions, including saliva, IgA is found exclusively as dimers that are bound to each other by the secretory component. The secretory component of IgA and IgM serves several physiological roles, including acting as glue to bind secreted IgA and IgM to the mucus layer overlying the gut epithelium, where they can bind to and neutralize gut pathogens and their toxic products. The secretory component of IgA also protects these antibodies against proteolytic cleavage enzymes produced by many pathogens.

Therefore, secretory IgA is capable of functioning as a blocking antibody, which can create a barrier to certain dietary macromolecules, bacteria, and viruses. The interaction with secretory IgA will not permit such antigens to interact with the mucosa and blocks their entrance into the circulation, leading to the prevention of autoimmune diseases.

Since saliva is a non-invasive medium and its analysis has been useful in measuring a wide range of antibodies, hormones, drugs, microbial, viral and fungal RNA or DNA, it has consequently become an easy and reliable biomarker in disease diagnostics.

Based on these facts, we have produced a single test that will inform the physician of important clinical conditions required to diagnose and to treat etiologically patients who may suffer from overgrowth of aerobic and anaerobic bacteria, candidiasis, food allergies or intolerance, celiac, Crohn's, ulcerative colitis, and many autoimmune diseases affecting the skeletal muscle, pancreas, kidney and brain. This patented test was developed because oral and intragastric exposure to dietary proteins first results in salivary IgA or IgM production, and then, under certain conditions, may result in IgA and IgG production in blood. This test was also developed since, in our experience, the diseases cannot be fully understood in their diagnostic and therapeutic implications without coordination of the other components of the intestinal flora and dietary antigens.

Conditions that adversely effect the intestinal flora can result in intestinal imbalance and enhanced gut permeability. Such reactions, when coupled with immuno dysfunction, can readily result in food allergies or intolerance, chemical hypersensitivity, candidiasis (candida related complex), some forms of Lupus and rheumatoid arthritis, asthma, chronic migraines, gastrointestinal disorders, as well as cellular immunosuppression.

In order to assist clinicians to make a more etiologically based diagnosis, we have developed the patented Intestinal Barrier Function Test (IBFT) for both blood and mucosal secretions. The IBFT utilizes a highly sensitive and accurate ELISA test method that measures the saliva IgA and specific antibody titers to the purified antigens from three aerobic (*E. coli*, *Lactobacillus* and *Enterococcus*), two anaerobic microbes (*Bacteroides fragilis* and *Clostridium perfringens*), *Candida albicans*, and dietary antigens such as egg, wheat, corn, soy and milk.

Such quantitative and comparative test results allow the clinicians to determine these primary clinical conditions: intestinal imbalance, enhanced gut permeability, humoral immunodeficiencies, candidiasis and many other related GI disorders and autoimmunities.

IBFT is recommended in patients who:

- have candidiasis that appears to be resistant to standard therapy
- are suspected of suffering from disturbances of intestinal permeability and absorption
- have complaints of food intolerance (including "food allergy")
- present as diagnostic problems with multiple symptom complaints (including chronic fatigue syndromes)
- or suffer from abnormal immune cell count and function (including autoimmune diseases)

Furthermore, in clinically asymptomatic Crohn's disease patients, an abnormal IBFT precedes clinical relapse by one year. Thus, it helps doctors to evaluate their patients for the loss of intestinal barrier function, based on which they can design new therapeutic strategies such as the use of probiotics, glutathione, lipoic acid, EDA/DHA and others aimed at re-establishing the intestinal barrier function.

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