ARRAY 14

ARRAY 14 – Antibody

Multiple Mucosal Immune Reactivity Screen™

Cyrex™ Laboratories

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CLINICAL APPLICATION GUIDE TO MUCOSAL IMMUNE REACTIVITY SCREEN™

MUCOSAL VERSUS SYSTEMIC IMMUNITY

There are two major mechanisms of protection in the body, both of which defend against foreign materials:

- Mucosal immune system
- Circulatory or systemic immune system

Both systems are independent of one another but have the capability to communicate with each other.

A. **The mucosal immune system** is the first line of defense that protects our body against the variety of antigens that comes in contact with the human body. Normally, these antigens are originated from the environment, oral cavity, lungs, and gastrointestinal (GI) tract. This system's major antibody isotype is IgA, which plays an important role in the elimination of food and bacterial antigens from the GI tract. The secondary antibody isotype is IgM, which, in individuals with IgA deficiency, compensates for the decrease in IgA function.

B. **The circulatory or systemic immune system** produces IgG, IgM and IgA isotype antibodies against antigens that come in contact with macrophages or antigen-presenting cells. Under certain conditions, for example, immune dysregulation, the body may produce IgE antibody, which is responsible for the prevention of parasitic infection or the induction of allergic reactions.

The major role of the IgA/IgM antibodies produced in saliva is their binding to food and bacterial antigens or toxic chemicals that manage to bind to proteins in the oral cavity or GI tract. Secretory IgA binds to these foreign substances to prevent their entry into the submucosa and the circulation, where immune reaction against them may result in multiple autoimmune reactivities. Repeated exposure to food antigens, bacterial toxins, and neoantigens originated from the GI tract results in overproduction of secretory IgA/IgM antibodies against various antigens in saliva. Furthermore, the binding of the antibodies to the antigens results in the formation of immune complexes.

The formation of these food, bacterial toxin, and neoantigen immune complexes may contribute to the failure of oral tolerance, the activation of inflammatory cascade, and the unwanted penetration of food, bacterial toxins and neoantigens into the submucosa and circulation. In addition, immune complexes that are formed by the binding of food antigens, bacterial toxins (LPS, BCDT) or neoantigens to their specific antibodies can bind to a very specific IgA receptor called CD71 on the surface of epithelial cells. This binding of antigen-antibody formations, for example, gliadin-IgA complexes or LPS-IgA complexes, to the IgA receptor may promote the entry of gliadin, LPS or other peptides or antigens through transepithelial transport into the submucosa and into the blood. The transport of intact peptides through the intestinal epithelium perpetuates inflammatory and immune responses against the penetrating antigens, resulting in the production of the cytokine IL-15 plus IgA and IgM in the saliva, as well as the possible production of IgG or other antibody isotypes if the intact antigens or peptides reach the blood.

Note that the production of antibodies in saliva and in blood against dietary proteins, bacterial toxins and neoantigens is possible in the absence of a leaky gut condition. Since the mucosal and circulatory immune systems are two separate entities, antibodies in saliva and serum may or may not correlate with each other; it is therefore possible for IgA antibodies against soy or egg to be detected in the saliva but not in the serum of one individual, and for antibodies against LPS to be detected in the blood but not in the saliva of another. Therefore, practitioners should not automatically expect the same antibody results in saliva and in blood. Overall research shows only about 50% correlation between IgA antibodies in saliva versus serum.

A Drop in the Bucket

Immune reactivity does not always cross systems. At times the mucosal immune system may be upregulated against a specific antigen, while at other times the systemic immune system will react. On occasion, both systems may react to the same antigen.

OVERVIEW

Home to 70% of the immune system, the mucosal immune system acts as the primary host defense against the physical environmental factors (food, airborne molecules, viruses and commensal antigens), and plays a significant role in barrier functions. Mucosal immunity is the main functional defense mechanism in urinary, respiratory, and the gastrointestinal systems. The intestinal mucosal interface is a complex system that must integrate interactions among the microbiota, biofilms, mucus layer, associated protective compounds, defensins, enzymes, secretory IgA, epithelial physiological interconnections, and underlying immune cells of the lamina propria. Notably, it has become clear that both the state of the microbial community and underlying immune cells contribute to the health or disease of the host. Secretory immunoglobulins IgA and IgM are important components of the first line of defense that operates at all mucosal sites.

A Drop in the Bucket

Mucosal immune reaction to an antigen results in the production of SIgA and/or SIgM antibodies in secretions including saliva.

The Gastrointestinal Mucosal Immune System

Protecting the intestinal barrier is the mucosal layer. Within the mucosa is the non-specific barrier, which is comprised of bacteria, gastric acid, mucus, defensins, and enzymes. Below the non-specific barrier is the specific immunologic barrier, home of secretory IgA (SIgA) see Figure 1 for a depiction of the mucosal immune system. The mucosal immune system contains more than 80% of all immunoglobulin-producing cells in the body, and the major product of these cells in normal individuals is SIgA.[1] SIgA blocks bacterial adherence, and prevents trans-mucosal entry of many xenobiotics, and potential carcinogens that contact mucosal surfaces.[2]

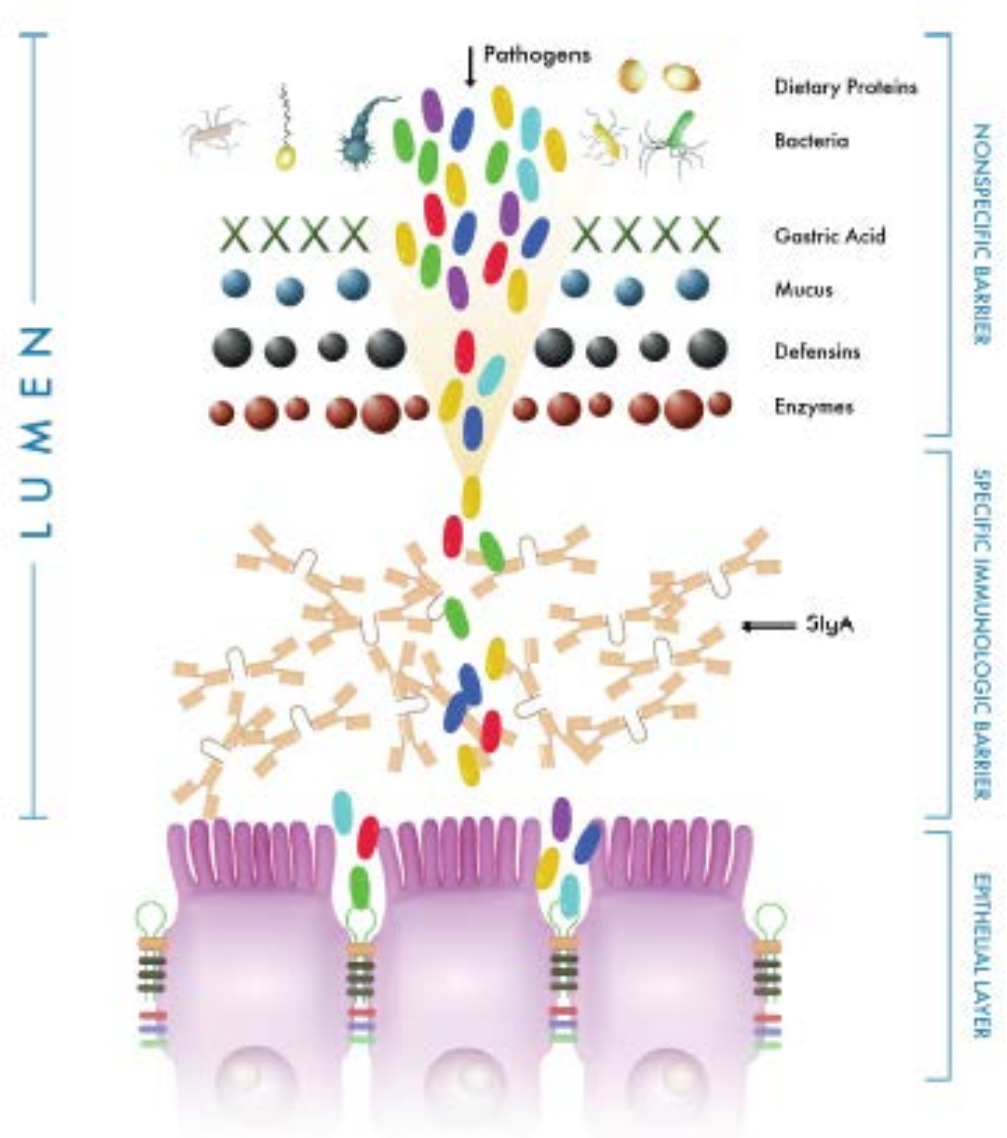

***Figure 1*. The mucosal immune system.** *Protecting the intestinal barrier is the mucosa, which has layers of components that break down environmental proteins and neutralize them before they can infiltrate the body.*

Saliva is a mucosal body fluid with important functions in oral, gastrointestinal and general health.[3] Oral fluid and its contents of the parotid, submandibular, and sublingual glands, with hundreds of minor salivary glands and gingival crevice fluid, helps clean the mouth, digest food and fight tooth decay. It also carries many of the same proteins and other molecules found in blood and urine. So far as part of proteomes of human saliva, scientists discovered more than 2000 proteins and associated proteins in human parotid and submandibular gland.[3 4] This catalogue of the salivary proteome of healthy individuals is helping in the detection of changes in some of these proteins in association with various disorders.[5 6]

A Drop in the Bucket

Over production of SIgA and/or SIgM antibodies against various antigens in saliva could be an indication of a defect in the oral tolerance mechanism, which can result in inflammatory conditions in the gut.

Studies have shown that oxidative stress,[7] parasitic infection,[2] breast cancer,[8 9 10 11] food sensitivity,[12 13 14] gluten reactivity,[15] Celiac disease (CD)[15 16 17 18] and inflammatory bowel disease,[19] leave identifiable and specific fingerprints in saliva. The immune reactions to antigens occur due to a breakdown in immune oral tolerance and subsequent inflammation and tissue damage or autoimmunity.[20 21]

Because the mucosa is the first line of defense, elevated salivary antibodies to the gut microbiota, gut-associated antigens, dietary proteins, xenobiotics and enteric nervous system (ENS) antigens assessed in Array 14 may be a warning of mucosal immune reactivity. If this specific mucosal immune reactivity is not addressed, the intestinal barrier may be breached by the environmental insult. If environmental toxins infiltrate the body, the resulting inflammation may eventually lead to the onset of autoimmune or neuroautoimmune disorders.

A Drop in the Bucket

Repeated exposure to an antigen in the mucosa that crosses the intestinal barrier results in antibody production in the blood.

The Enteric Nervous System

The gut has its own special brain called the enteric nervous system (ENS). The ENS is located in sheaths of tissue lining the length of the gastrointestinal system from esophagus to colon. It is the largest and most complex division of the peripheral and autonomic nervous systems (PNS and ANS) in vertebrates.[22]

In the developing human gut, enteric neural crest cells complete their rostro-caudal migration between weeks 4 and 7 of development.[23] Despite the presence of enteric neural crest cells earlier in gestation, the development of gut motility does not occur until late gestation or after birth.[24] [25] The reason for the significant time lag between the appearance of neurons within the gut, and the establishment of neural control of gut motility has yet to be elucidated.[22]

The ENS is home to numerous different types of neurons close in number to that of the spinal cord.[22] During the 19th Century German scientist Leopold Auerbach (1828-1897) identified a complex network (plexus) of nerve cells and fibers wedged between two layers of muscle encircling the gut.[26] This myenteric plexus has been named the Auerbach's plexus and contains both parasympathetic and sympathetic properties. Originating in the medulla oblongata as a collection of neurons from the ventral part of the brain stem, the myenteric plexus contains the neurons that regulate enzyme output of the gall bladder and pancreas.[26] Gastrointestinal motility is controlled by the myenteric plexus. Within the submucosa, just beneath the lining of the gut's internal cavity, lies a second network of intestinal nerve tissue. This submucosal plexus has been named Meissner's plexus, which provides secretomotor innervation. This layer contains sensory cells that communicate with the deeper myenteric plexus and motor fibers that stimulate the secretions of fluids in the lumen.[26] See Figure 2 for a depiction of the ENS.

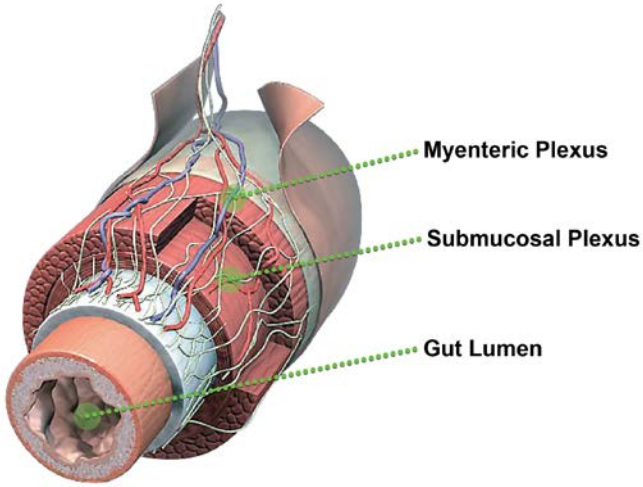

Figure 2. Small intestine cross-section. *The ENS lines the intestines in sheaths that house the submucosal plexus (Meissner's plexus) and the myenteric plexus (Auerbach's plexus).*

The ENS provides various functions that control:

- motility of the intestine
- exchange of fluids across the mucosal surface
- blood flow
- secretion of gut hormones.[22]

Humans have more nerve cells connected to our gastrointestinal system, than there are in the spine. The gut-brain axis clearly exists. More research is needed to identify and better understand this special and fascinating connection between the gut and the brain.

CLINICAL SIGNIFICANCE

Using saliva in the clinical setting offers several advantages over alternatives, not only because it is a non-invasive and cost-effective method, but in time is also proving to be of higher values in terms of accuracy.[27] The detection of mucosal immune reactivities against various environmental and tissue antigens associated with many disorders can be used to improve clinical outcomes. Recent developments in genomic, proteomic, and metabolomic approaches have facilitated sensitive and high‐throughput testing methods for saliva and are proving increasingly useful for diagnostics.[27]

IgA and IgM antibody production against various antigens in saliva may be an indication of a defect in oral tolerance which can result in inflammatory conditions in the bowel. Mucosal or oral tolerance is the suppression or downregulation of immune effect of cell responses, either T- or B-cell, to an antigen by prior exposure of the antigen by mucosal route. Defects in the mechanism of oral tolerance have been reported as being responsible for several diseases of the gastrointestinal and respiratory tract in particular gastric autoimmunity.[21 28 29 30 31 32] Impaired mucosal immune reactivity resulting from malnutrition displays the inability to regulate and exclude the mucosal flora and dietary and environmental antigens, which causes an increase in the incidences and severity of infection, local inflammation and tissue damage, and possibly increases the individual's susceptibility to allergy, autoimmunity, and neoplasia.[2]

After repeated exposure of mucosal immune cells to antigens and the subsequent production of IgA + IgM in the mucosal secretions, these antibodies then interact with many dietary proteins, resulting in immune complex formation, which further contributes to the inflammatory reaction in the gastrointestinal tract.[33 34 35 36 37 38] Based on this mechanism of action, saliva is a source of body fluid for detection of an immune response (90% IgA and 10% IgM) to bacterial, food, xenobiotic, and other antigens present in the oral cavity and gastrointestinal tract. Indeed, salivary antibody induction has been widely used as a model system to study secretory responses to ingested material, primarily because saliva is easy to collect and analyze.

PATHOPHYSIOLOGY (MECHANISMS OF TISSUE DAMAGE)

A recent observation outlines the capacity of secretory IgA immune complexes to promote the retrotransport of intact peptides across the intestinal epithelium.[39] The role of a defective epithelial barrier may be to promote the entrance of exogenous peptides through transepithelial transport.[40 41 42 43] It seems that dietary antigens, are complexed to intraluminal secretory IgA that is produced against them.

A Drop in the Bucket

After repeated exposure of mucosal immune cells to antigens and subsequent production of high levels of SIgA and/or SIgM, these antibodies then interact with many dietary proteins and bacterial toxins, forming immune complexes, which contribute to inflammatory reactions in the gastrointestinal tract.

For example, gliadin peptides complex with secretory IgA bind to the IgA receptor, which then transports and protects them from lysosomal degradation through a specific transcytosis pathway,[41] (see Figure 3) thereby perpetuating the immune inflammatory responses, which result in the production of IgA, IgM and cytokines in oral fluid.

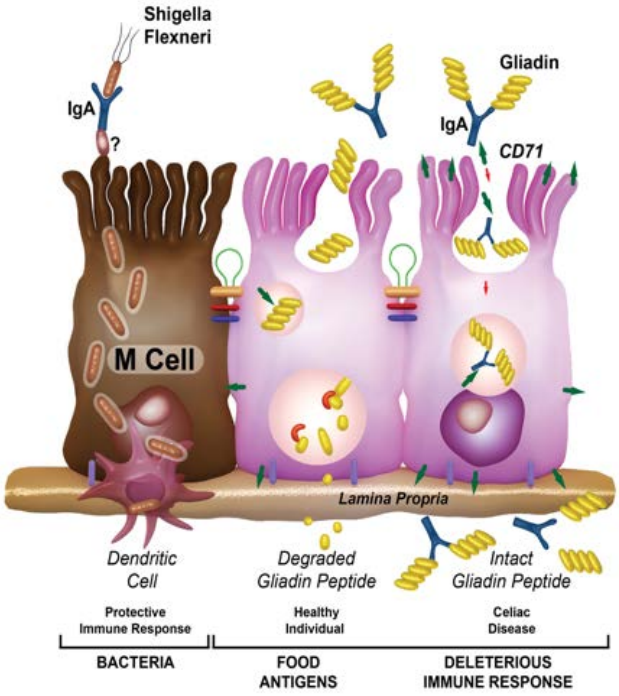

Figure 3. Immunoglobulin A-mediated retrotransport of luminal food antigens. *Gliadin antigens complex with SIgA. The complex binds to the IgA receptor on the epithelial cell, which then transports the complex into the cell through a specific transcytosis pathway leading to the lamina propria and eventually systemic circulation.*

Since the mucosal immune system is a central component of host defense, as a whole, any dysregulation and inflammatory reaction in the GI tissue may result in intestinal barrier dysfunction and the entry of digested, or undigested, dietary proteins into the circulation. Dietary proteins in the circulation result in systemic immune response and the production of very high levels of IgG and IgA against dietary proteins and peptides.

This breach of the intestinal barrier by dietary proteins, and other molecules, due to loss of tolerance not only can lead to IgG and IgA production in blood, but also might lead to an immune response to different target organs and the induction of autoimmune diseases.[29 44 45 46 47 48 49]

Environmental triggers may have the capacity to affect the tight junctions. Such triggers may include bacterial antigens, viral antigens, mold antigens, xenobiotics, dietary components, and associated tissue antigens. The antibodies can react with their specific antigens and form immune complexes, which further contribute to the entry of antigens, immune complexes, or other inflammatory molecules into the submucosa, and then into circulation. Due to structural similarity between the intestinal barrier and blood-brain barrier (BBB), these IgA + IgM antibodies, immune complexes and inflammatory signals can also affect the integrity of the BBB resulting in autoimmunity against nervous system tissues.

A Drop in the Bucket

Immune complexes have the capacity to promote transport of intact peptides across the intestinal epithelium, using very specific receptors even in the absence of a broken intestinal barrier (leaky gut).

INFLUENCING FACTORS

The triad concept of autoimmunity consists of three important components often present for the development of an autoimmune disease: 1) genetic background, 2) environmental components, and 3) gut and BBB permeability.

Genetic

Selective IgA deficiency (SIgAD), a condition in which a person makes normal levels of immunoglobulins except for IgA, is one of the common primary immunodeficiency diseases.[50] Many individuals remain relatively healthy and are never diagnosed with the disease, while others can have significant illnesses. Autoimmunity (see Figure 4) occurs in about 25-33% [50 51 52] and allergies or asthma occurs in 10-15%.[53 54] SIgAD is found more frequently in males than females.[55] SIgAD was found in 155 of 72,296 blood donors; furthermore, HLA typing of 62 unrelated IgA deficient blood donors showed a significant increase in the prevalence of HLA-B8 (p less than 0.005).[56]

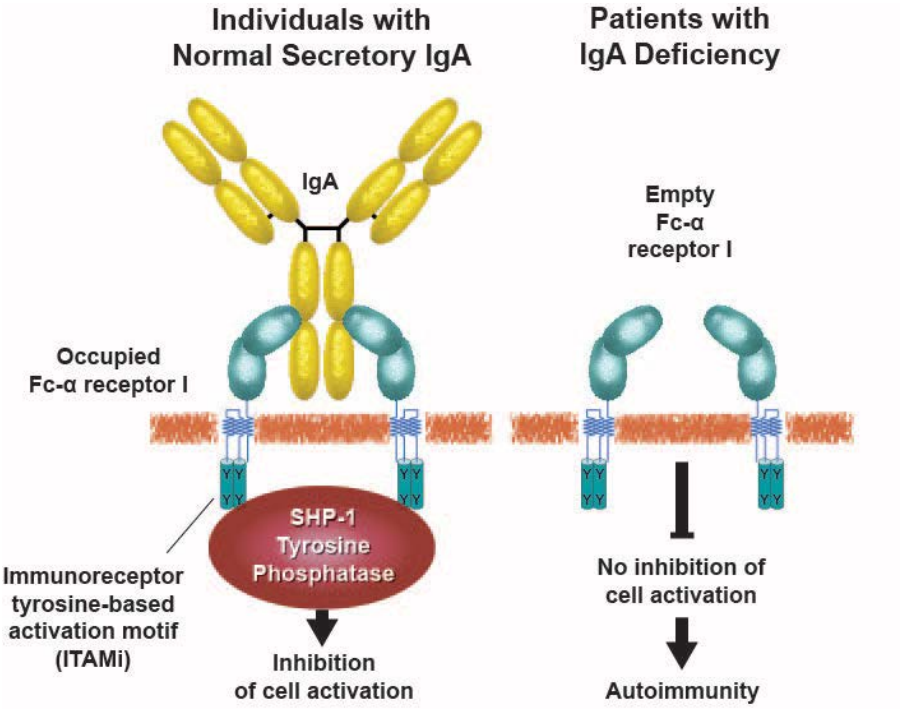

Figure 4. Model Illustrating the Facilitating Role of FC-alpha Receptor I (FcαRI) for Autoimmunity in Patients with IgA Deficiency. *FcαRI acts as a regulator, which mediates both anti- and pro-inflammatory functions of IgA depending on the type of interaction.*

Deficiency of IgA is thought to be present from birth in most cases. There is an increased frequency of infections in SIgAD that could, theoretically, trigger autoimmune disorders such as Grave's disease and systemic lupus erythematosus.[57] On the other hand, in cases of Celiac disease, SIgAD has occasionally been reported to occur after the onset of the gastrointestinal symptoms, thus a common genetic background is likely to be the main contributor to the autoimmune disorders in which environmental factors determine if, and when, the primary and secondary diseases will appear.[57]

Although genetic and environmental factors both play a central role in autoimmunity, many times it is not clear which one is the main link to heterogeneity of autoimmune prevalence. The importance of genes in autoimmunity became emphasized when it was noticed that the risk of autoimmunity is increased in twins and siblings of affected individuals.[58] Gene analysis studies thereafter have confirmed the genetic relevance and suggested different methods for predicting the development of autoimmune conditions such as systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus type 1, and multiple sclerosis on an individual basis.[59 60 61 62]

Environmental

A variety of environmental factors that can affect mucosal immune reactivity and initiate immunological inflammatory cascades.
Environmental factors influencing inflammatory cascades include:

- Stress
- Gut dysbiosis
- Infections
- Dietary proteins
- Chemical toxicity

Medical History

Risk factors for mucosal immune dysregulation include:

- Smoking
- Alcoholism
- Chemotherapy and head/neck radiation
- Protein-energy malnutrition (PEM)

MEASURING MUCOSAL IMMUNE REACTIVITY

Due to the predominance of IgA in secretions, most clinical laboratory antibody assessments are measuring SIgA only. One of the contributors of low levels of secretory IgA is chronic stress (see Table 1), which is rampant in many industrialized countries. Thus measuring SIgA alone has the potential for many false negatives. However, it is known that SIgM is increased when SIgA is suppressed.[34 63 64] Therefore, to increase sensitivity of salivary antibody testing at Cyrex Laboratories, SIgA and SIgM are measured simultaneously. Cyrex also requires patients collect unstimulated saliva. Studies show that unstimulated saliva secretion contains at least three times more IgA than the stimulated counterpart.[63 65 66]

A Drop in the Bucket

Many factors can influence the production of secretory IgA. For most patients with suppressed SIgA production, SIgM compensates. Therefore, the patient still maintains mucosal immune protection.

Table 1. Conditions that can contribute to increased or decreased level of secretory IgA.

Increased Secretory IgA Level	Decreased Secretory IgA Level
Acute stress	Chronic stress
Some medications	Some medications
Oropharyngeal carcinoma	Adrenal insufficiencies
Chronic oral infection	Bacterial colonization on molar surfaces
Chronic GI infection	Recurrent tonsillitis
Heavy smoking	Adenoid hyperplasia
Alcoholism	Cutaneous candidiasis
Periodontitis	Asthmatic with recurrent respiratory tract infection
Dental plaque accumulation	Intense endurance exercise
Intestinal barrier dysfunction	Intestinal barrier dysfunction
Celiac and Crohn's diseases, Ulcerative colitis	Celiac and Crohn's diseases, Ulcerative colitis
	Recurrent herpes infection
	Nutritional deficiencies

Assessing Total Secretory IgA (SIgA)

TEST	RESULT			
Total Secretory IgA	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Total Secretory IgA	1.3			0.2-1.7

Total SIgA resulting within the reference range, as shown above, not below the reference range (< 0.2) is ideal. Results below the reference range (<0.2) indicate mucosal immune suppression of immunoglobulin A. Generally, the immune system compensates the suppressed SIgA by making more SIgM. The increase in SIgM will provide the patient with mucosal immune protection despite the lower SIgA levels. Results above the reference range, as shown below, indicate mucosal immune up-regulation.

TEST	RESULT			
Total Secretory IgA	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Total Secretory IgA			2.50	0.2-1.7

Since the mucosal immune system is a central component of host defense, as a whole, any dysregulation and inflammatory reaction in the GI tissue may result in intestinal barrier dysfunction and the entry of digested dietary proteins into the circulation. Dietary proteins in the circulation result in systemic immune response and the production of very high levels of IgG and IgA against dietary proteins and peptides.

This breach of the intestinal barrier by dietary proteins due to loss of tolerance not only can lead to IgG and IgA production in blood, but also might lead to an immune response to different target organs and the induction of autoimmune diseases.[45 67 68 69 70 71 72 73 74 75 76 77 78 79]

CLINICAL USE OF CYREX ANTIBODY ARRAY 14

This Array is a very cost effective, easy, and non-invasive method, to measure mucosal immune reactivity to a range of exogenous and endogenous antigens. The categories of antigens include assessments for intestinal dysfunction, food immune reactivity, gut dysbiosis, infection, and possible enteric nervous system autoimmune reactivity. This mucosal immune response is the body's first immune system reaction. If steps to bring balance back to the mucosal immune response are not taken, the immune reactivity may result in a breach of the intestinal barrier, followed by a systemic immune reaction. The inflammation of systemic autoantibodies contributes to the progression of autoimmune and neuroautoimmune reactivities.

Mucosal screening with Array 14 has the following advantages:

- Non-invasive specimen collection
- Assesses the unique mucosal reactions to an array of gut-related environmental antigens
- Identifies an early event in immune reactivity to an array of gut-related environmental antigens
- Measures immune reactivity at its earliest stage for potential Celiac disease, non-celiac gluten-sensitivity, irritable bowel disease, *etc*.
- Identify gut dysbiosis
- Monitor effectiveness of dietary protocols and other interventions
- Cost effectively assess multiple antigens at once

Array 14 can be used to:

- Evaluate mechanisms of compromised immune tolerance.
- Evaluate possible outcomes of compromised mucosal tolerance, such as: intestinal barrier dysfunction, food and chemical immune reactivity, and autoimmunity.

Array 14 is recommended for patients who:

- Have chronic inflammatory bowel conditions.
- Have a family history of autoimmune disease.

CLINICAL INTERPRETATION FOR ANTIBODY ARRAY 14 – MUCOSAL IMMUNE REACTIVITY SCREEN

Individual mucosal reactivity results may vary. Table 2 shows the percent positives (out of range) on 257 clinical specimens assessed at Cyrex.

Table 2. Antigen Percent Positive on 257 Patient Results.

Array 14 Antigen	% Positive	Array 14 Antigen	% Positive
Lipopolysaccharides	24.9	Egg	12.45
Occludin/Zonulin	8.95	Soy	21.79
Actomyosin	11.28	Corn	13.23
ASCA + ANCA	18.68	Alpha-Casein + Beta-Casein	15.18
Calprotectin	24.51	Casomorphin	19.07
Native + Deamidated Alpha-Gliadin-33-mer	11.28	Aflatoxin	14.79
		Bisphenol-A	11.67
Gamma-Gliadin-15-mer	14.01	Mercury	4.28
Glutenin-21-mer	7.78	Mixed Heavy Metals	5.84
Gluteomorphin	15.18	Rotavirus	15.56
Wheat Germ Agglutinin	21.4	Myelin Basic Protein	8.56
Transglutaminase-2	15.56	Blood-Brain Barrier Protein	6.23

Array 14 test results are not diagnostic for any clinical condition or disease. These reports may be used in conjunction with other pertinent clinical data for the purposes of diagnosis.

Table 3. Interpretation of Array 14 – Mucosal Immune Reactivity Screen. *A general guide to interpreting Array 14. Please make an appointment through our Customer Service Team to speak to a Cyrex Clinical Consultant for a more in-depth, individualized interpretation.*

ANTIGEN	CLINICAL SIGNIFICANCE OF SALIVARY ANTIBODIES
Lipopolysaccharides	Gut dysbiosis
Occludin/Zonulin	Beginning stage of autoimmune reactivity to intestinal barrier tight junctions
Actomyosin	Beginning stage of autoimmune reactivity to intestinal epithelial cells
ASCA/ANCA	Inflammation and activation of neutrophils in the GI tract
Calprotectin	Gastrointestinal inflammation
Alpha-Gliadin-33-mer	Mucosal gluten-reactivity
Gamma-Gliadin	Mucosal gluten-reactivity

ANTIGEN	CLINICAL SIGNIFICANCE OF SALIVARY ANTIBODIES
Glutenin	Mucosal gluten-reactivity
Gluteomorphin	Mucosal gluten-reactivity, lack of the enzyme DPPIV
Wheat Germ Agglutinin	Mucosal lectin sensitivity and activation of complement via lectin pathway
Transglutaminase-2	Beginning stage of autoimmune reactivity to intestinal villi
Egg	Mucosal egg immune reactivity
Soy	Mucosal soy immune reactivity
Corn	Mucosal corn immune reactivity
Alpha + Beta Casein	Mucosal dairy immune reactivity
Casomorphin	Mucosal dairy immune reactivity, lack of the enzyme DPPIV
Aflatoxin	Mucosal immune reaction to dietary aflatoxin
Bisphenol-A	Mucosal over-exposure to BPA and its binding to salivary proteins
Mercury	Mucosal over-exposure to mercury through diet and/or dental amalgams, and its binding to salivary proteins
Mixed Heavy Metals	Mucosal over-exposure to heavy metals through diet and their binding to salivary proteins
Rotavirus	Chronic exposure to Rotavirus and induction of inflammation in the gut, which can contribute to Celiac disease or non-celiac gluten sensitivity
Myelin Basic Protein	Beginning stage of immune reactivity to enteric nervous system, or possible cross reaction between dietary proteins and peptides and infectious agents with enteric neurons
Blood-Brain Barrier Proteins	Release of S100B from activated macrophages and adipocytes, and early immune reactivity against S100B, the major component of the blood-brain barrier
Immune Complexes	Antigen-antibody immune reaction and activation of complement via the lectin pathway in the gastrointestinal tract, which promotes the transport of undigested food antigens and bacterial toxins across the intestinal epithelium in the absence of a broken intestinal barrier (leaky gut)

ANTIGEN	CLINICAL SIGNIFICANCE OF SALIVARY ANTIBODIES
Majority of Array 14 Antigens Positive	Polyreactivity Polyreactive antibodies are antibodies that are produced in response to certain antigens (see below), but which react not only with the antigens against which they were produced but also to many unrelated antigens, including self-tissues. Although these antibodies are produced against one antigen, their role is to protect the body against directly related and many unrelated antigens. Polyreactive antibodies produced in saliva act as a barrier against the entry of commensal microbes, pathogens, and undigested food antigens into the submucosa and circulation. Very often polyreactive antibodies are detected in saliva and in blood in the absence of apparent disease, but the presence of these antibodies indicates that the mucosal immune system has been exposed to one or more of the antigens that originate from bacteria, viruses, food, or xenobiotics. The human body's immune system produces these polyreactive antibodies to create an umbrella of antibody protection against these antigens as well as autoantigens in order to prevent autoimmunities in the future. For example, our research has shown that antibodies produced against wheat germ agglutinin, soy agglutinin, or peanut agglutinin have the capacity to react to 50-132 completely different food antigens, 15-20 different bacterial/viral antigens, and 8-18 different human tissue antigens such as islet, cell, α-myosin, and thyroid peroxidase.

IMMUNE POLYREACTIVITY

When a person has highly elevated antibodies against all, or nearly all, antigens on a large antibody test panel, the antibodies are referred to as polyreactive antibodies. Polyreactive antibodies (PAbs) are antibodies that are produced against a particular antigen or antigens, but can bind to a variety of different and structurally unrelated self- (Fc fragment of IgG, insulin, thyroglobulin, ssDNA) and non-self- (bacteria, viruses) antigens with simple (haptens) or complex (carbohydrates, proteins, nucleic acids, lipids) structure.[80] Because of their flexibility, PAbs can be a normal product of the immune system or they may become pathogenic, depending on conditions.[81]

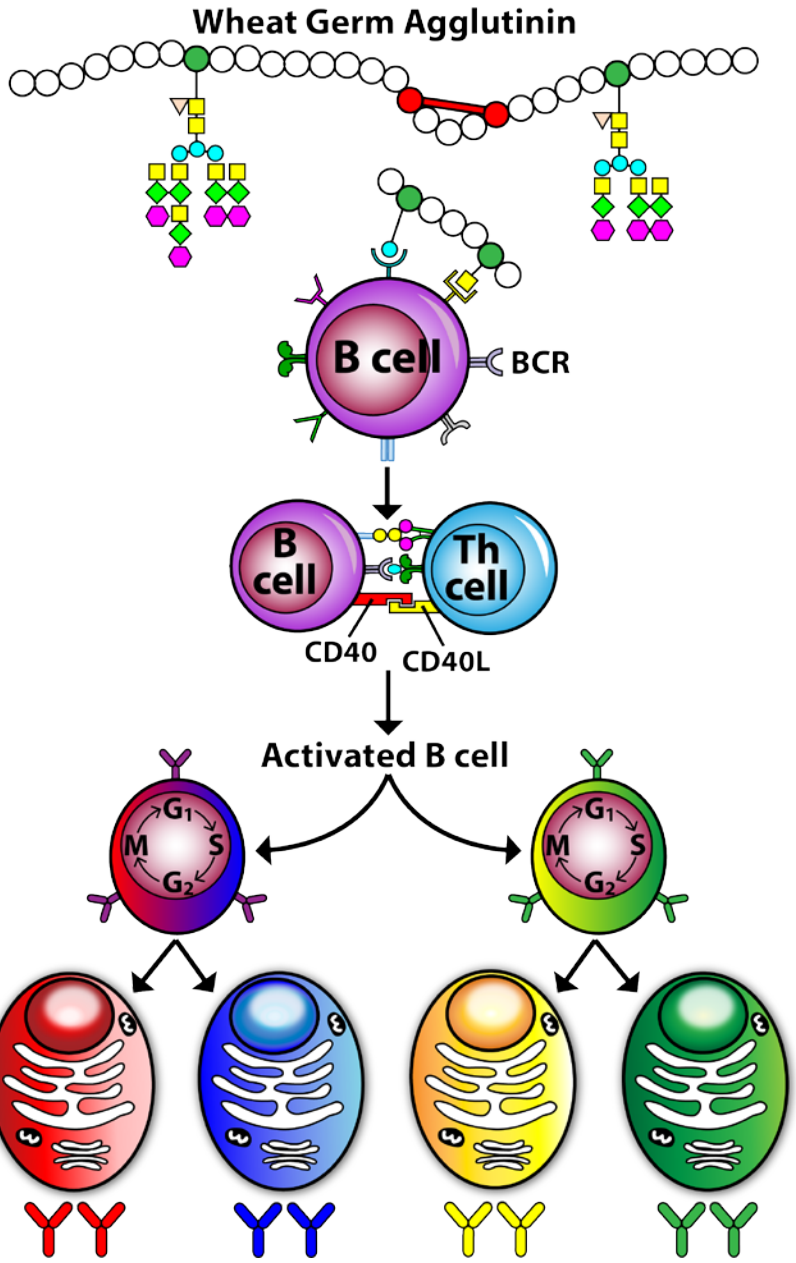

Figure 5. The Binding of Wheat Germ Agglutinin to B-cell receptors (BCR) Initiates Signals that Alert Th Cells for Collaboration. *The T-helper cell releases cytokines that induce B-cell activation and differentiation into plasma cells. Plasma cells produce not only antibodies reacting to specific food antigens such as lectins, but also some that will react to many other unrelated food and even tissue antigens. This is why these antibodies are called polyreactive. Each color represents a different antibody.*

PAbs were discovered in the early 1980s. Research ensued to elucidate the function of these special antibodies (see Figures 5 and 6). It was concluded that PAbs are cleared from the circulation substantially faster than monoreactive antibodies,[80] which explains why many polyreactive patients live inflammation-free, healthy lives.

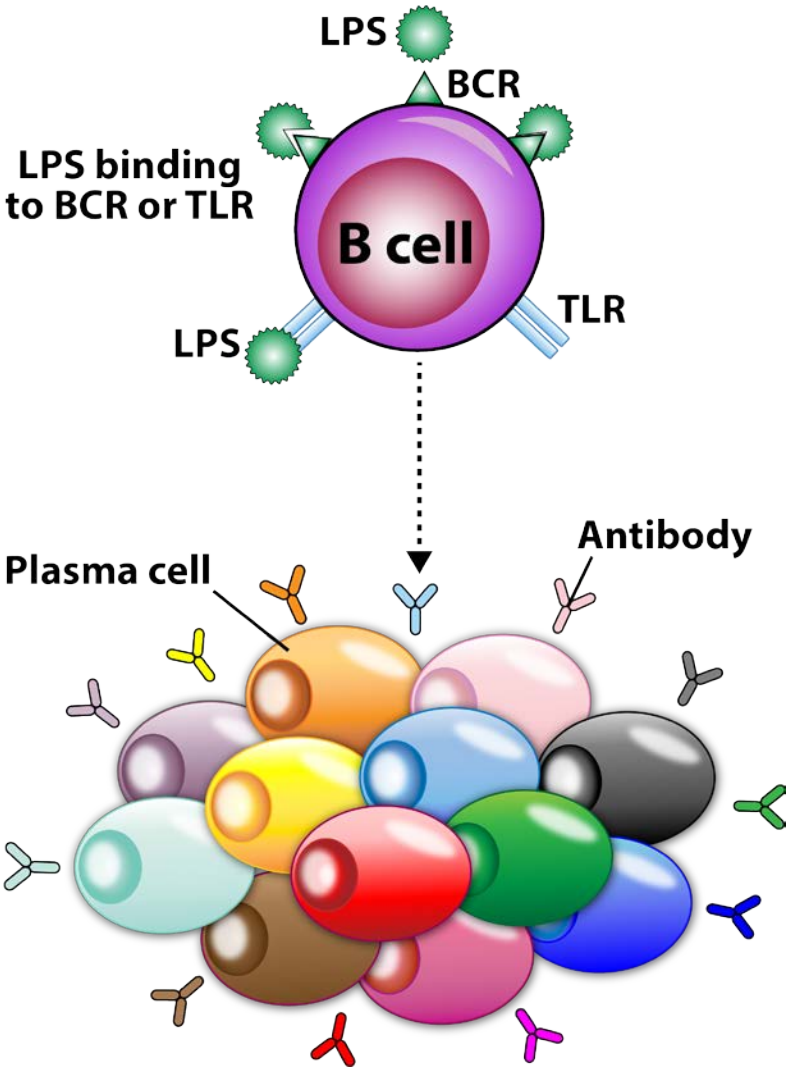

Figure 6. Polyreactive Antibody Formation. *Binding of lipopolysaccharides (LPS) to toll-like receptor (TLR) and B-cell receptor (BCR) in the induction of more diverse polyclonal/polyreactive immunoglobulin repertoire production. Each color represents a different antibody.*

In health, polyreactivity of immune receptors magnifies the antigen detection power of the immune system and endows the system with the ability to exert regulation of its own functions.[81] These circulating PAbs are low-affinity antibodies that do not damage self-tissue.[82] If conditions remain in this state, a person producing PAbs will remain healthy.

A Drop in the Bucket

Polyreactive SIgA blocks bacterial adherence and blocks trans mucosal entry of undigested food antigens, bacterial toxins and some xenobiotics.

On the other hand, the presence of PAbs has been associated with different autoimmune, inflammatory, and infectious diseases. PAbs can break immune tolerance and cause the formation of immune complexes as seen in autoimmunity; PAbs can stimulate cells as seen in allergy; PAbs can even contribute to malignancy by the perpetual malignant cell stimulation.[81] It is still not clear whether, or not, PAbs play a direct role in immune disorders leading to disease. In cases of infection, PAbs can assist the cell infiltration of certain pathogens,[81] and low-affinity PAbs have been shown to switch to high-affinity in HIV patients.[83]

Not all polyreactive patients have health problems. However, they are at greater risk for developing health problems, if the polyreactive patient's body encounters a traumatic event. Acute physical (infection, car accident) or emotional (death of loved one) stressors can switch natural polyreactivity to a state of high-affinity binding. For others, the first immune response can be low-affinity PAbs, later followed by high-affinity, in pathogenesis progression.[84]

Certain conditions can cause a person to produce PAbs.

- Pathogens, including periodontal pathogens, which can result in multiple positives on Array 14.
- Microbe involved in biofilm formation.
- Bacterial toxins such as lipopolysaccharides and cytolethal distending toxin-B
- Viral antigens, notably Epstein-Barr virus early antigen and EBNA.
- Human and bacterial heat shock proteins.
- Molds and mycotoxins, especially *Stachybotrys* and Satratoxin
- Some vaccines containing viral or bacterial antigens, toxic chemicals and food proteins.
- Chemicals such as DNP-BSA, food coloring and other neo-antigens.
- Medications with specific chemical structures that directly or indirectly form neo-antigens with human tissue.
- Bioactive nanoparticles containing chemicals, gelatin, dextran and human albumin.
- Lectins/Agglutinins, especially peanut and bean agglutinins.
- Seaweeds that contain a very high percentage of lectins which are glycoproteins or N-glycopeptides.
- Microbial transglutaminases (food/meat glue)
- Apoptotic cell components, nuclear antigens and ssDNA
- Various gums and oleosins
- High fat diet
- High salt diet

Is it good or bad to be among the roughly 5% of the population that produces secretory polyreactive antibodies?

- It's good because polyreactive antibodies are produced in order to bind to a variety of xenoantigens from food, gut bacteria, and neoantigens so as to clear these antigens from our system and prevent harmful immune reactivity.
- It's bad because the production of polyreactive antibodies is an indication of overexposure to one or more of the antigens shown in Table 1, such as lectins, agglutinins, gums, EBV or Rota virus.

Most research on immune polyreactivity has been done with serum immunoglobulins, however, there is a small body of polyreactivity research done in mucosal secretions,[85] [86] showing that polyreactivity can occur in both the mucosal and the systemic immune systems. At Cyrex, about 5% of tested individuals produce polyreactive mucosal antibodies. Below is an example of a mucosal polyreactive test result.

TEST	RESULT			
Array 14 - Multiple Mucosal Immune Reactivity Screen	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Lipopolysaccharides IgA+IgM			1.94	0.2-1.8
Occludin/Zonulin IgA+IgM			2.72	0.2-2.0
Actomyosin IgA+IgM			3.77	0.2-2.0
ASCA/ANCA IgA+IgM			>3.80	0.2-1.9
Calprotectin IgA+IgM			2.68	0.2-1.8
Alpha-Gliadin-33-mer IgA+IgM			3.38	0.2-2.0
Gamma-Gliadin-15-mer IgA+IgM			2.80	0.2-2.0
Glutenin-21-mer IgA+IgM			2.85	0.2-2.0
Gluteomorphin IgA+IgM			2.27	0.2-2.0
Wheat Germ Agglutinin IgA+IgM			2.79	0.2-1.9
Transglutaminase 2 IgA+IgM			2.15	0.2-1.9
Egg IgA+IgM			2.61	0.2-2.1
Soy IgA+IgM			2.85	0.2-2.0
Corn IgA+IgM			2.49	0.2-2.3
Alpha + Beta Casein IgA+IgM			2.41	0.2-2.0
Casomorphin IgA+IgM			2.49	0.2-2.0
Aflatoxin IgA+IgM			2.38	0.2-2.1
Bisphenol-A IgA+IgM			3.47	0.2-2.2
Mercury IgA+IgM			3.30	0.2-2.3
Mixed Heavy Metals IgA+IgM			2.91	0.2-2.1
Rotavirus IgA+IgM			2.80	0.2-2.1
Myelin Basic Protein IgA+IgM			2.84	0.2-2.1
Blood-Brain Barrier Proteins IgA+IgM		2.22		0.2-2.4
Immune Complexes IgA+IgM			2.68	0.2-2.0

How can we confirm that results indicate polyreactive antibodies?

In general, but not always, research shows that if an individual reacts to several neoantigens formed as the result of the binding of toxic chemicals and his immune system produces detectable IgM antibodies against them, then the patient is most likely generating polyreactive antibodies.

Therefore, if you have tested your patient for IgM antibody against different haptenic chemicals bound to human serum albumin, such as those in Cyrex's Chemical Immune Reactivity Screen (Array 11), and a majority of them turned positive, then your patient is among a very small percentage of the population that makes high levels of polyreactive antibodies.

Some polyreactive patients do not have immune reactivity to chemicals bound to human tissues.

SPECIMEN REQUIREMENT

4 mL oral fluid*
Ambient

*Studies show that unstimulated saliva secretion contains at least three times more IgA than the stimulated counterpart.[63 65 66] Do not chew paraffin strips to stimulate saliva secretion.

RELATED TESTING

- **Antibody Array 2 – Intestinal Antigenic Permeability Screen (Serum)**
- **Antibody Array 3X – Wheat/Gluten Proteome Reactivity and Autoimmunity (Serum)**
- **Antibody Array 4 – Gluten-Associated Cross-Reactive Foods and Foods Sensitivity (Serum)**
- **Antibody Array 7 – Neurological Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 7X – Expanded Neurological Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 11 – Chemical Immune Reactivity Screen (Serum)**
- **Antibody Array 12 – Pathogen-Associated Immune Reactivity Screen (Serum)**
- **Antibody Array 20 – Blood-Brain Barrier Permeability Screen (Serum)**
- **Antibody Array 22 – Irritable Bowel / SIBO Screen (Serum)**

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