ARRAY 10-90

90 Real-World Food Antigens

MULTIPLE FOOD IMMUNE REACTIVITY SCREEN™

COOKED • RAW • MODIFIED

CYREX® Laboratories

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CLINICAL APPLICATION GUIDE TO MULTIPLE FOOD IMMUNE REACTIVITY SCREEN™

OVERVIEW

There is an increasing awareness that foods may play a much larger role in immune reactivity than previously thought. We all eat food, and foods are a constant and sustained source of antigens, much more so than other things we may be exposed to.

Food Immune Reactivity (FIR) is rapidly increasing in prevalence for reasons that remain unknown. Current research activities are focused on understanding the immunological basis by which environmental factors contribute to this phenomenon.[1] [2] [3] Advancements in the field of mucosal immunology have provided many clues about the role of environmental triggers, in particular the role of toxic chemicals (xenobiotics) and disturbances in the gut microbiota as risk factors in the development of food immune reactivities.[4] [5]

Currently, most laboratories test for foods either by cellular cytotoxic assay based method, or IgG or IgA antibody based method, and associate their test results with food allergies and sensitivities. The cellular cytotoxic method is not supported by the peer reviewed medical literature and is not considered to be a reliable medical diagnostic tool; since it has not been appropriately validated, it is not a suitable guide for therapeutic decisions.[6] [7]

According to the Australasian Society of Clinical Allergy and Immunology:[8]

> *"These results have been shown to not be reproducible, give different results when duplicate samples are analyzed blindly, don't correlate with those from conventional testing, and 'diagnose' food hypersensitivity in subjects with conditions where food allergy is not considered to play a pathogenic role."*

Cyrex is addressing a much larger issue. The IgG and IgA immune reactions to food proteins and the chemicals in foods potentially causing disorders that already affect over 53 million Americans.

Autoimmune diseases are on the rise and foods may be partially responsible.

Autoimmune diseases are the third most common category of disease in the United States after cancer and heart disease.[9] Autoimmune diseases have not been this prevalent before; they are 3 times more common now than they were just a few decades ago. And this is not due to increased recognition or better diagnostic criteria. It is due to the fact that more people are getting autoimmune diseases than ever before. And the numbers keep climbing. One in six Americans will develop an autoimmune disorder – and one in four women. This pandemic includes rheumatoid arthritis, multiple sclerosis, Crohn's disease, lupus, and type I diabetes mellitus. Genetics play a role, albeit not a major one: approximately 1/3 of the risk for autoimmune diseases is due to hereditary factors according to the National Institutes of Health. The majority, around 70%, is due to environmental factors.[9] Exposure to chemicals, including those in foods and foods antigens, gut dysbiosis, and infections, are the main causes of autoimmune disorders.

What is the link between chemicals in foods and autoimmune diseases?

Most foods we consume contain chemicals, even when they are labeled organic. Food production uses chemicals as preservatives, additives, dyes, flavorings, colorings, and for texturing. Food contact materials, such as packaging materials, conveyer belts and tubing materials at factories and plants, and even the tubes of the espresso machine through which the coffee passes all leach chemicals into foods. Agriculture today uses chemicals in the form of artificial fertilizers and herbicides, insecticides, fungicides and other pesticides.

Take a food, wheat for example, which the immune system recognized as a "friendly protein" early in a person's life when they first ate bread, thereby developing oral tolerance. Oral tolerance is the immune system not reacting or being unresponsive to the oral ingestion of an innocuous antigen such as a food protein. So why now, suddenly, does this person, as well as almost 25% of the population, react to wheat protein, which is found in bread, pasta, and many other foods? Because there is a new chemical attached to the wheat: it could be a pesticide, or a fungicide, or an insecticide, each having the potential to form a new antigen after being attached to wheat. Also, due to hybridization, new peptides have been introduced into wheat that were not there previously. For these reasons, the immune system won't recognize this wheat as "friendly" when these other chemicals are attached to it. Instead, it detects the new antigens, made up of the wheat and the chemical/s, and attacks the ingested wheat (**see Figure 1**). As the molecular makeup of these food antigens sometimes resemble the molecular sequence of a tissue, the body's immune system now begins to attack the self-tissue antigens, launching an autoimmune reaction, later potentially developing into an autoimmune disease unless preventive measures are taken under the direction of a clinician.[5 10 11]

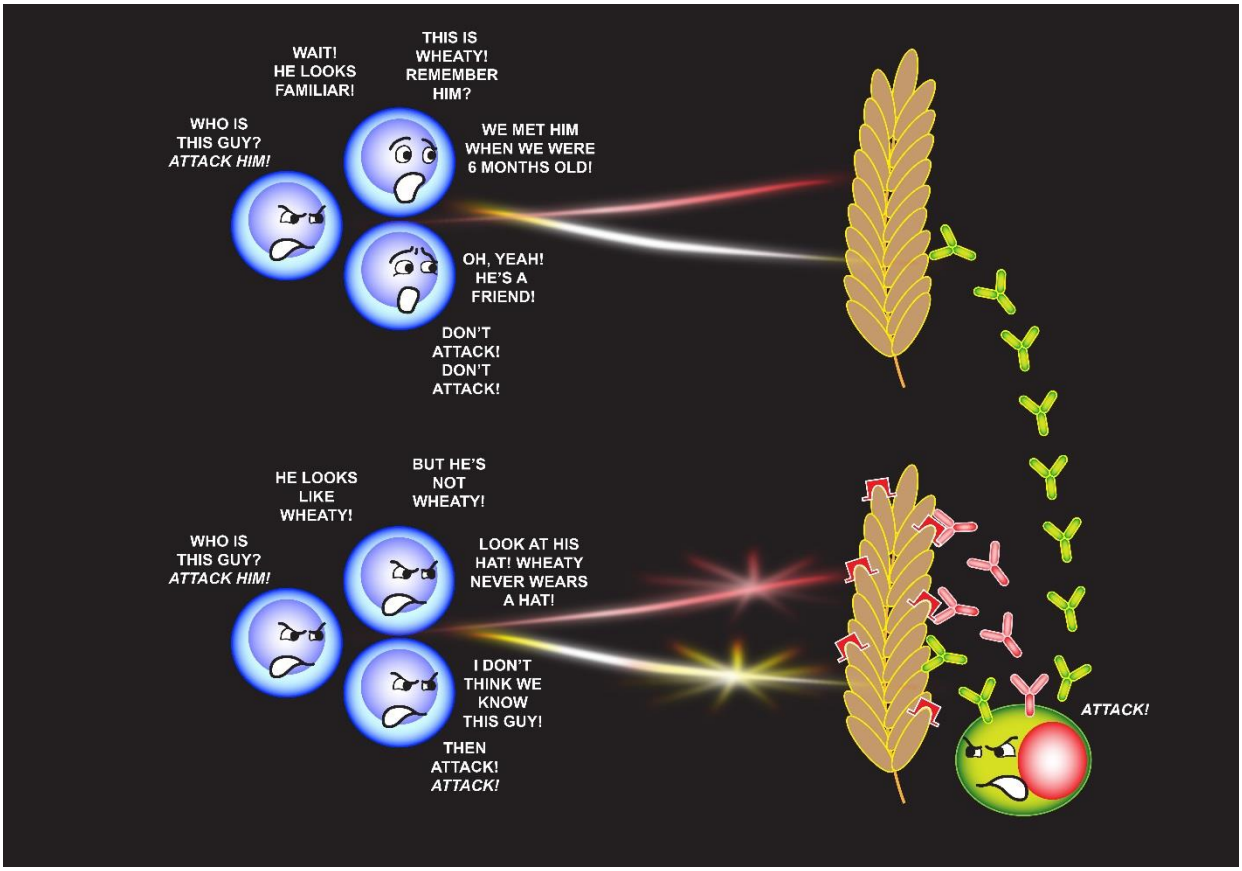


Figure 1. Breakdown in oral tolerance due to contamination of dietary components by toxic chemicals. An initial exposure primes the immune system to recognize a food antigen as being non-threatening, resulting in oral tolerance. However, if the immune system is exposed to that same food antigen bound to chemicals (hats), it may not recognize the chemical-bound food as friendly food and therefore react adversely to it.

What is important for the clinician to know?

The Cyrex Difference

Cyrex has developed a new way of testing for food immune reactivity. This method arises from a foundation of science and medicine. **Array 10-90 – Multiple Food Immune Reactivity Screen™** features 10 unique characteristics that set Cyrex apart from other laboratories.

1. *Raw and Cooked*

Array 10-90 assesses immune reactivity to raw and cooked food proteins. This reflects how foods are most commonly eaten. This is necessary because when food is heated or cooked, its protein structure changes. The foods being assessed should best duplicate what patients eat. Cyrex is the only laboratory to test for both raw foods which are eaten raw and cooked foods that are eaten cooked. An example of how heat changes the

integrity of food proteins is shown in **Figure 2**. Note the change in color of both the yolk and egg white in proportion to the length of cooking.

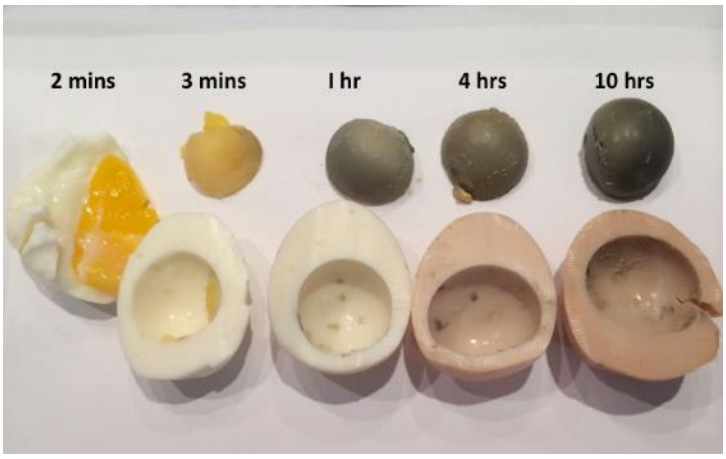


Figure 2. Effect of heat and time on egg proteins. Food proteins change when heated. Egg protein has been shown to change depending on length of cooking time.

2. *Cross-Reactive, Pan-Antigen Isolates*

Specific food antigens are known to cross-react with human tissues. If a person makes antibodies to these specific food antigens, and the person has barrier permeability, those antibodies to the specific food antigen can begin attacking human tissue. This can result in tissue damage, autoimmune reactivity and eventually autoimmune disease. Some cross-reactive food antigens include, gliadin, casein, food aquaporin and shrimp tropomyosin. Pan-antigens are proteins that are common among multiple sources. An example of pan-antigens is shrimp tropomyosin. Tropomyosin is found in a variety of fish and crustaceans, which has been shown to cross-react with human tropomyosin.

3. *Multiple Food Protein Interactions*

When food proteins are combined during processing, the antigenicity of the individual food proteins can change. In other words, a patient may not react to canola oil, raw potato, or event baked potato, but when the potato is fried in canola oil, the patient may react to the fried potato. Real-world diets include combined foods; some are obvious like fried potato, while some are hidden as in the case of meat glue. In Array 10-90, we assess combined food proteins including meat glue, wheat + alpha-gliadins, dark chocolate, tofu, and fried potatoes.

4. *Large Gum Molecules*

Gums are in many foods, especially gluten-free and dairy-free processed products. They can also be found in soups, juices, jams, salad dressings, soy products, dairy products such as milk and yogurt, and others. Gums are large molecules (200,000-5,000,000 Daltons) and parts of their molecules have the same molecule sequences as other food

proteins; this is known as molecular mimicry. These can cross-react with other food proteins, causing an immune reaction in the patient.

5. *Binding Isolates (Lectins and Agglutinins)*

Lectins are glycoproteins that bind carbohydrates, and agglutinins bind cells together. Lectins and agglutinins are found in about 30% of foods. Lectin is only one among hundreds of proteins found in beans, so it is normally not possible to accurately measure the lectin antibody when it is mixed with many other proteins. However, by using purified lectins, the most antigenic protein in beans, peanuts, etc., the testing becomes the most accurate and specific method to detect antibodies to these inflammatory food antigens. Array 10-90 includes beans agglutinins.

6. *Tissue-Bound Artificial Food Colors*

Artificial food colorings are used extensively in foods, and humans are regularly exposed to them by ingestion. These chemical colorants form adducts (bonds or "bridges") with proteins in humans; therefore, measuring the antibodies to these colorants will indicate whether or not they are responsible for a patient's immune or autoimmune reaction. A patient may not react to a particular food; however, they may react to the food once its protein is bound with an artificial colorant. It is important to note that we are talking about food proteins binding to artificial food colorants, and vice-versa. The binding of artificial colorants to a food protein may increase the food's antigenicity and ability to cause an enhanced immune reaction in patients.

7. *Amplified Antigenic Proteins and Peptides*

Array 10-90 includes specific proteins and peptides that are within the entire food proteins. Examples include shrimp tropomyosin and shrimp protein, bean agglutinins and whole bean protein. These antigens are highly purified recombinant proteins (proteins made via biomolecular engineering) and synthetic peptides (short chains of amino acids). By targeting specific antigens within the entire food proteins, Array 10-90 increases the sensitivity and specificity for food immune reactivity.

8. *Oleosins*

Cyrex tests for oleosins, which are the oil proteins found in seeds and nuts. Some patients may not have a reaction to the proteins in seeds or nuts such as sesame, peanuts, and others; however, they may react to the protein oil in a seed or nut. This is why a patient may react to both sesame and sesame oleosins, or may have a reaction only to sesame oleosins. In the latter case testing only for sesame and not sesame oleosins would give a false negative.

9. *Meat Glue*

Meat glue, also known as transglutaminase or thrombian, is a powder used in the food manufacturing industry to adhere smaller pieces of meat to make one large fillet, or to turn flakes of white fish into imitation crab meat, or form chicken scraps into nuggets. It is also used to thicken some milks, yogurts and egg whites. According to the packaging

label on meat glue, there is also maltodextrin and sodium caseinate with transglutaminase.

10. *Dual Antibody Detection System*

Because both IgG and IgA isotypes are involved in the immune response, Array 10-90 measures IgG and IgA antibodies for each food item. Clinically, IgA is an indication of the mucosal immune response, and IgG is an indication of the circulatory immune response. By measuring both, this insures and enhances the detection of food immune reactivity.

In addition to the 10 Point System, Array 10-90 also incorporates Cyrex's Core Quad of Standards, which ensures highly accurate results.

Core 1 – *Antigen Purity Technology*

Each food goes through a biochemical purification process so that only the purest form is tested. This ensures the purity of each food antigen and the reliability of the results. A test is only as good as the purity of the antigen used.

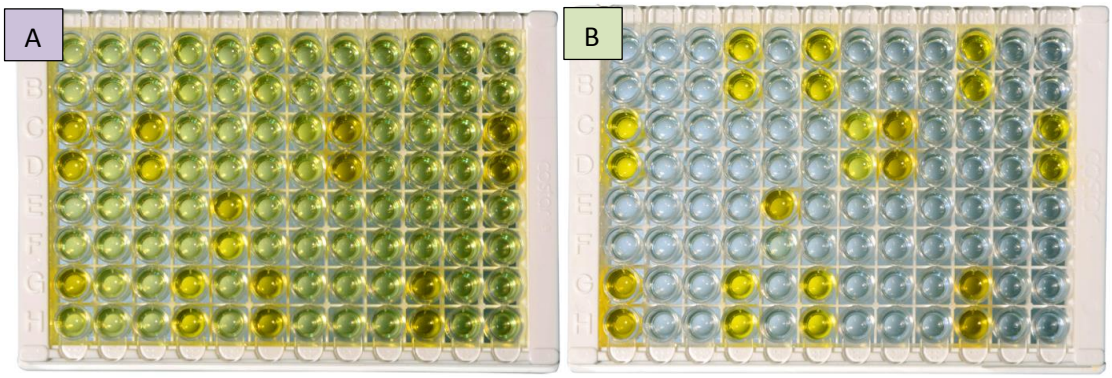


Figure 3. Purified antigen results versus non-purified antigen results. Plate A was coated with non-purified food antigen, while Plate B was coated with purified food antigen. The purified food antigen yielded fewer positive results, as only the food protein was included on the plate.

Core 2 - *Optimized Antigen Concentration*

Optimized antigen concentration along with antigen purity ensures accuracy, precision and the analytical sensitivity and specificity of the test. Using apple as an example, apple protein concentration is 0.2%, meaning in 100 grams of apple, there are 200 mg of protein; parsley has the same proportion of protein per 100 grams. On the other hand, almond has 20% protein, meaning in 100 grams of almond, there are 20,000 mg of protein, or 100 times more apple and parsley, as is shown in **Figure 4**.

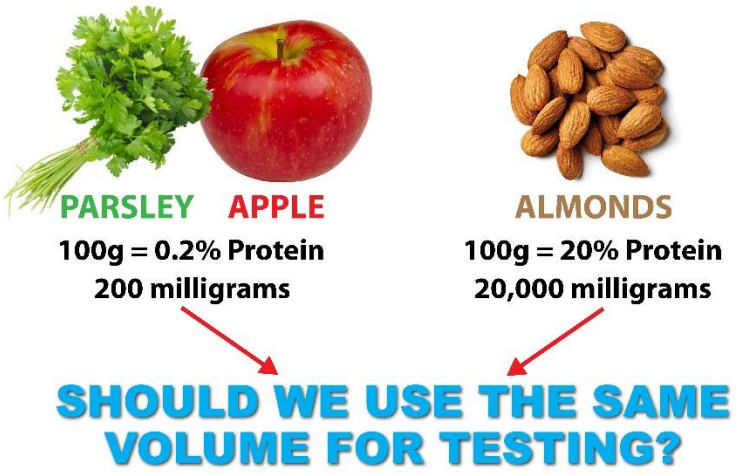


Figure 4. Difference between protein concentrations in parsley, apple and almond. Determination of proper antigen concentrations for testing needs to take percentage of protein into consideration for optimal results.

Unfortunately, the practice of using equal volumes of each food to be tested, regardless of the protein concentration, has continued to be used ever since IgG food testing was developed in 1985. Today, Cyrex goes through several steps to purify the food antigens and then painstakingly establishes the optimal concentration of each food for testing.

Core 3 – *Parallel Testing (Duplicate Testing)*

To ensure correlation and accuracy of the results, each patient blood sample is run twice, as is shown in **Figure 5** below. If there is a lack of correlation between any of the side by side duplicate or parallel measurements, the patient specimen is run in parallel quadruplicate. Every test result released by Cyrex shows reproducibility by double testing for each antibody against any tested antigens.

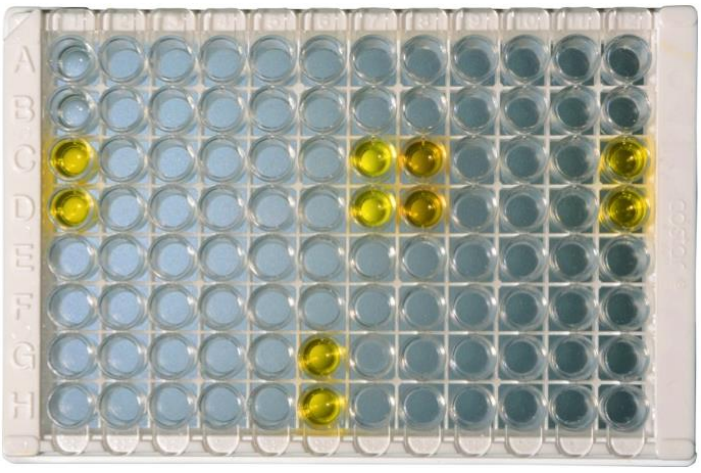


Figure 5. Example of double testing. In parallel testing, a specimen is placed on the plate in side-by-side wells. There must be correlation between the side-by-side well

results. If there isn't correlation, the specimen needs to be re-run. Results will be released only when the lab can show correlation with the side-by-side wells.

Core 4 – *Antigen Specific Validation*
The Federal Clinical Laboratory Improvement Amendments of 1988 (CLIA-88) regulations at 42CFR493 and the FDA state:

"All assays that are introduced into the clinical laboratory must have established and verified method performance specifications before patient testing is performed."

More specifically:

"Prior to reporting patient test results, the laboratory must verify or establish, for each method, the performance specifications for the following characteristics: accuracy, precision, analytical sensitivity and specificity; the reportable range of patient test results; the reference ranges; and any other applicable performance characteristic."

Unfortunately, the practice of validating sets of foods against different antigen has continued to be used ever since IgG food testing was developed in 1985. Cyrex takes extra steps, to ensure accuracy of test results, each food antigen is validated against its own standard rather than in sets. The Cyrex way increases sensitivity and specificity over simply applying the standard of wheat or milk to tomato, beef, almond, mint and a host of other food antigens.

In a Nutshell

By implementing the above 10 practices, Cyrex offers a unique food immune reactivity test, that can provide better clinical outcomes.

MECHANISMS OF FOOD INDUCED AUTOIMMUNITY

The Gastrointestinal Mucosal Immune System as Related to Foods

A child is born with almost no protective immune system other than passive immunity and maternal transfer of IgG against various food antigens and infectious agents. Although the child is born germ-free and with no microbiota in the GI tract, the mucosal membranes are bombarded immediately after birth by a large variety of microorganisms originating first from the mother; secondly from handling by the doctor and nursing personnel, then from breast milk or commercial formula, and lastly, from exposure to various food antigens upon the introduction of solid food. For this reason the mucosal immune system has evolved two arms of adaptive defense to handle these challenges.

The mucosal immune system is the first line of defense against environmental triggers such as dietary components, chemicals and microbes. One of the principal elements of the mucosal immune system is the acquisition of oral tolerance by which the immune system learns not to react to food antigens and friendly microbiota.[12] For this reason the gut mucosa is made of the largest collection of lymphoid tissue in the body, including the largest proportion of activated and regulatory lymphocytes. It is this non-stop communication between these activated lymphocytes and regulatory T-cells that determines immunological homeostasis, which is crucial for the overall well-being of an individual. Therefore, exposure to environmental factors such as food proteins and infection is a natural and necessary thing that has a physiological role in the maturation of the immune system both locally and systemically, but exposure to toxic chemicals is not.

Changes or disturbances in the mucosal immune system and the gut microbiome have been associated with food immune reactivity. Studies indicate that in the state of balanced microbiota, specific bacteria and their products provide immune protection.[13] In mouse studies, mice with reduced commensal bacteria colonies in their gut, including antibiotic-treated or germ-free mice, showed increased food immune reactivity.[14] [15] Environmental factors such as toxic chemicals (Array 11), bacterial toxins, such as lipopolysaccharides (Array 2), food additives (Array 10, Array 10-90), medications, and undigested proteins and peptides (Arrays 3, 4, 10 and 10-90) can induce failure of oral tolerance, promote gut permeability and systemic food immune reactivity.[5] [16]

In a Nutshell

In the gastrointestinal tract, the immune system's first line of defense is the mucosal immune layer. If this layer becomes dysfunctional it puts the intestinal barrier at risk for breach by food antigens. This could result in loss of immune tolerance to these food antigens.

Array 10-90 can be used to:

- Evaluate immune reactions to foods, raw and/or modified, food enzymes, lectins and artificial food additives, including meat glue, colorings and gums.
- Early detection of dietary-related triggers of autoimmune reactivity.
- Monitor the effectiveness of customized dietary protocol in your patient.

Array 10-90 is recommended for patients who:

- Seek a life-long health and wellness strategy.
- Present with unexplained symptoms whether gastrointestinal, neurological, dermatological or behavioral in nature.
- Are suspected of having increased intestinal permeability, which is the gateway for environmentally-induced autoimmune disorders.

Immune Tolerance

Immune tolerance is the immune system's ability to differentiate between what is harmful and what is safe. The immune system is engineered to react to viruses, pathogenic bacteria and other foreign antigens, while not attacking self-tissue, food proteins/peptides and commensal bacteria. Tolerance refers to the specific immunological non-reactivity to an antigen resulting from a previous exposure to the same antigen through cell deletion or immune suppression mechanism.[12]

One of the important jobs of the immune system is to protect the body against antigens from the outside world. Thus, the immune system must be able to discern "self" from "non-self." It is supposed to recognize self-tissue as "self" and leave it alone, while at the same time identify and attack non-self-antigens such as viruses, parasites, and xenobiotics if bound to antigens and food proteins. When this system is working, it is called immune tolerance.[5 17] When immune tolerance is lost, inflammation and autoimmunity can occur depending on the individual. A variety of factors known to contribute to loss of immune tolerance are summarized in **Table 1**.

Table 1. Factors involved in oral tolerance induction and disturbance in this mechanism

• Maternal exposure to xenobiotics
• Mother's diet
• Canal birth versus C-section
• Breast feeding versus formula
• Baby formula or protein hydrolysate formula
• Time of the introduction of solid food (exposure to food proteins after weaning)
• Gut microbiota and its source
• Integrity of digestive enzymes
• Use of drugs or medications
• Genetics of the host

Oral tolerance is induced by multiple cellular and molecular processes to insure lack of immune reactivity to harmless intestinal derived antigens both in the mucosa as well as in the systemic immune system.[18] Together mucosal and circulatory induced tolerance appears to prevent intestinal disorders such as inflammatory bowel disease, food immune reactivity, and organ-specific and non-specific autoimmunities. This process is done by a very special population of dendritic cells (DCs) found in the microenvironment of mesenteric lymph nodes. The presence of antigen-specific T-cells and nodes and cytokines, such as TGF-β and IL-10, induce the generation of differentiation of these DCs into $FOXP3^{+}$ regulatory T-cells. These committed T regs home back to the intestinal lamina propria, where some of them may exit from the mucosa via the lymphatic system or blood stream and disseminate throughout the immune system promoting systemic oral tolerance.[17 18]

The ability of oral tolerance to maintain an inhibitory environment by the T reg cells and the production of non-inflammatory IgA against both dietary proteins and microbiota in secretions can prevent hyperimmune reactivities in the mucosa and in circulation.[19 20 21] The perinatal period is therefore crucial for the establishment of oral tolerance and to the induction of food immune reactivities.[22] Food immune reactivities can be due to many environmental factors that can disturb the homeostasis of the immune system, resulting in the penetration of dietary proteins and non-tolerogenic peptides to the sub-mucosa.

To avoid immune reactivity to food antigens, the body employs immune defenses, including secretory IgA (SIgA) antibodies and hyperesponsiveness to innocuous agents, particularly dietary antigens and the commensal gut microbiota.[23 24 25 26] The induction of these homeostatic mechanisms depends on exogenous stimuli, and the neonatal period is particularly critical to this end. Both the intestinal surface barrier with its reinforcement by SIgA and the immunoregulatory network require adaptation.

In most cases this adaptation is remarkably successful in view of the fact that a ton of food, which may include 100 kg of proteins, may pass through the gut of an adult human being every year without causing adverse reactions. Food immune reactivity reflects a lack of such homeostasis due to persistently imbalanced immune regulatory network. Breakdown of this homeostasis may be associated with immune reactivity, in particular IgG and IgA production against food proteins.

In a Nutshell

Tolerance refers to the specific immunological non-reactivity to an antigen resulting from a previous exposure to the same antigen. Loss of immune tolerance leads to immune activation. If the loss of tolerance is to self-tissue, this is the beginning of autoimmune reactivity.

How do you get from eating food to developing an autoimmune disease? How can our immune system attack ourselves when it is supposed to be protecting us? What is the mechanism? First, we must mention some obvious facts. The foods we eat today are very different from the foods our grandparents and their ancestors ate for thousands of generations. We use artificial fertilizers, which are mostly chemicals; we spray our crops with chemical pesticides, fungicides and insecticides; we liberally use antibiotics and hormones in cattle, chicken, turkey, and swine; we line juice cartons, milk cartons, cans, etc. with chemicals that leach into the food or beverage; we eat foods containing chemical colorants, chemical preservatives, and chemical flavorings; we use plastic containers for many beverages, as well as juices, oils, etc. We microwave foods in plastic containers. Most of our foods are processed foods. All of this adds up to chemicals in the foods, which then bind to food antigens. Furthermore, we cook differently than our ancestors: we use microwave ovens, coated pots and pans, and different types of heating elements on our stovetops. And the price we pay for all this is that the composition of our foods is so changed that our bodies, more specifically our immune system, no longer recognize these as friendly foods and treats them as an invader, creating chronic inflammation and setting the stage for autoimmune reactions.[11]

The mechanisms that may set the stage for food immune reactivity and increase the risk of developing inflammation are:[18]

1. Failure of oral tolerance.
2. Enhanced intestinal permeability to macromolecules.
3. Direct binding of food components to human tissue antigens.
4. Molecular mimicry and cross-reactivity between food antigen and human tissue as the major cause of autoimmunity.

Failure of Oral Tolerance

Failure of oral tolerance is the root cause in the pathogenesis of autoimmune diseases. If oral tolerance is lost, inflammation in the gut develops and it becomes more permeable to undigested proteins, which can then travel through the mucosal barrier to the submucosa, and from there enter the regional lymph nodes and into the circulatory system.[12] **Figure 6** below gives a visual and step-by-step demonstration of this process, which is also explained in the section "Immune Tolerance" above.

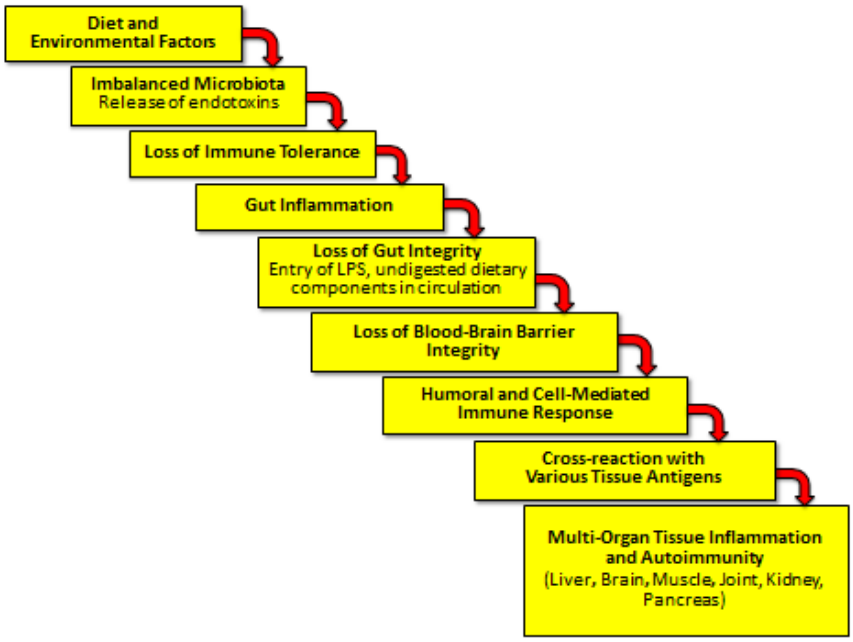


Figure 6. **How environmental factors can lead to failure of oral tolerance and subsequently to autoimmune disease.** Environmental factors can start a cascade of reactivities that may lead to autoimmunity.

Enhanced Intestinal Permeability to Macromolecules

The gut is presented daily with a multitude of foods that must undergo digestion, a process that starts with salivation in the mouth and continues through our gastrointestinal tract. We take proteins, break them down into peptides, and then further into amino acids; these are then absorbed in the gut. The process is supposed to work harmoniously: we eat and our system removes what it needs from foods and discards the rest. However, the reality is that stress, processed foods, lack of digestive enzymes, chemicals, medications, etc. alter this delicate harmonious balance by decreasing our ability to digest all the foods we eat. Therefore, our gut is frequently presented with undigested or partially digested foods in the form of undigested proteins and peptides. The bacteria in our gut, known as the gut microbiome, feeds on these undigested proteins and peptides, changing the gut microbiota. This change brings about the release of endotoxins called lipopolysaccharides (LPS) by these bacteria, causing inflammation and opening up the tight junctions, causing damage to occludin/zonulin, actomyosin and other cell junction proteins. This damage allows these proteins and peptide to cross the mucosal barrier, migrate into the regional lymph nodes and end up in the circulation.[27 28] The immune system is stimulated into action by 2 mechanisms:

1) some of these peptides bind directly to human tissues so the immune system attacks both the tissue and the peptides; 2) cross-reactivity between food proteins and human tissue amino acid sequences which, if left undetected, may develop into autoimmunity. To find out if your patient may have this problem, Cyrex Array 2 – Intestinal Antigenic Permeability Screen™ measures lipopolysaccharides (LPS), occludin/zonulin, and actomyosin antibodies.

Direct Binding of Food Components to Human Tissue Antigen

With today's fast-paced, can't-wait-for-it, gotta-have-it-now lifestyle, many kitchens in the US are stocked with pre-packaged, microwavable, heat-it-and-eat-it foodstuffs. To understand the ingredients lists of some of these foods, one needs an advanced degree in chemistry. **Figure 7** shows the ingredients from a single processed food product.

Ingredients
Sugar, Water, Corn Syrup, Enriched Bleached Wheat Flour [Flour, Reduced Iron, B Vitamins (Niacin, Thiamine Mononitrate (B1), Riboflavin (B2), Folic Acid)], Coconut (Sulfite Treated), Partially Hydrogenated Vegetable and/or Animal Shortening (Soybean, Cottonseed and/or Canola Oil, Beef Fat), High Fructose Corn Syrup. Contains 2% or Less of: Cocoa, Gelatin, Modified Corn Starch, Glucose, Sweet Dairy Whey, Leavenings (Sodium Acid Pyrophosphate, Baking Soda, Monocalcium Phosphate), Mono and Diglycerides, Soy Flour, Polysorbate 60, Soy Lecithin, Cornstarch, Salt, Soy Protein Isolate, Calcium and Sodium Caseinate, Sodium Stearoyl Lactylate, Dextrose, Cellulose Gum, Eggs, Natural and Artificial Flavors, Potassium Sorbate and Sorbic Acid (to Retain Freshness). Coatings Contain: Blue (FD&C Blue 1 Lake, Blue 2 Lake), Green (Yellow 5 Lake, Blue 1 Lake), Lavender (Blue 2 Lake, Carmine, Red 40 Lake), Orange (Yellow 6 Lake), Red (Red 40 Lake), Pink (Carmine, Red 40 Lake), Teal (Blue 1 Lake, Yellow 5 Lake), Yellow (Yellow 5 Lake).

Figure 7. Packaged Food Ingredients List. Few of these ingredients are whole food sources. Instead, this food product is made from a multitude of unnatural sugars, chemically-treated fractions, food colorings and artificial flavors.

Artificial food colorings, though known to cause DNA damage, adverse effects on the liver and kidneys, and have carcinogenic properties, have not been restricted but have actually seen increasing use in a growing number of foods for the last 50 years.[29 30 31 32]

According to the FDA website, there are two categories making up FDA's list of permitted colors:

- CERTIFIABLE: batch certification is required
- EXEMPT: batch certification is not required

A table can be found at:

http://www.fda.gov/ForIndustry/ColorAdditives/ColorAdditiveInventories/ucm115641.htm#table1B

Interestingly, the FDA official site states:

"FDA evaluates safety data to ensure that a color additive is safe for its intended purposes. Color additives that FDA has found to cause cancer in animals or humans **may not** be used in FDA-regulated products marketed in the United States"

Artificial food colorings are chemicals, called haptens. These molecules are too small for the immune system to recognize and mount an immune response against them. However, when chemicals enter the human body, they bind to various tissues and subsequently form neo-antigens. Immune reaction to these neo-antigens can result in a breakdown in immune tolerance and the production of antibodies against the tissues to which these chemicals bind.[4 33] Food colorings are generally ionic and thus they interact strongly with proteins to form covalent bonds, (**Figure 8**).[34] These stable complexes with proteins give uniform color distribution in all common food proteins.[39 40]

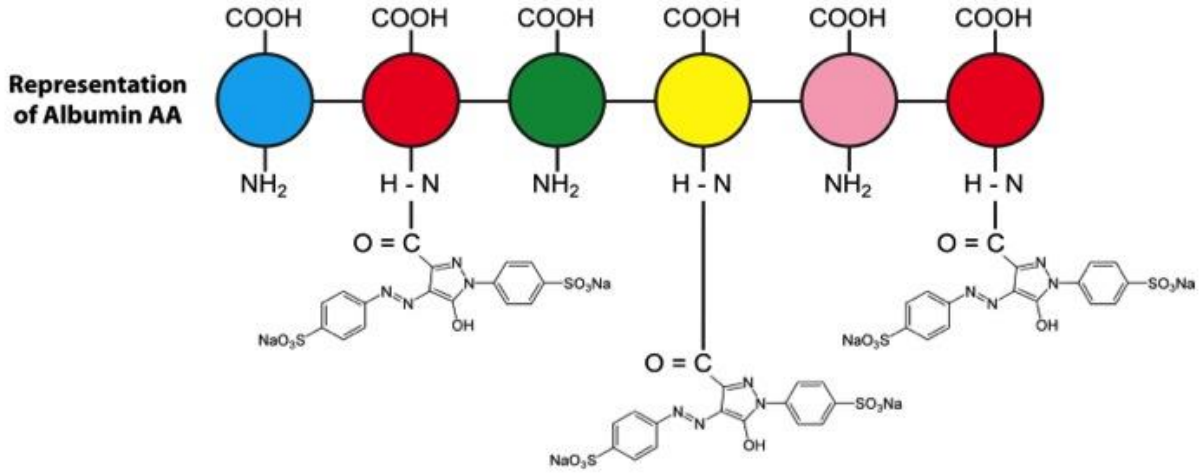


Figure 8. Covalent Binding of Food Coloring to Amino Acid Chain. Food colorings are generally ionic and thus they interact strongly with proteins to form covalent bonds.

Unfortunately, covalent binding of food colorings to human proteins, including human serum albumin and hemoglobin, [34 35 36 37] is a major mechanism for the induction of immune reactivity associated with various colorants.[38] Additionally, the covalent binding of food coloring to different food amino acid sequences prevents digestive enzymes from breaking down the food product[34 39] (**see Figure 9**). Artificial food colorings have significant immunological consequences due to their ability to bind to human tissues and/or prevent effective digestion.[40 41] Each of these events can activate the inflammatory cascade and result in food and food additives immune reactivities with a potential for autoimmune reactivity.

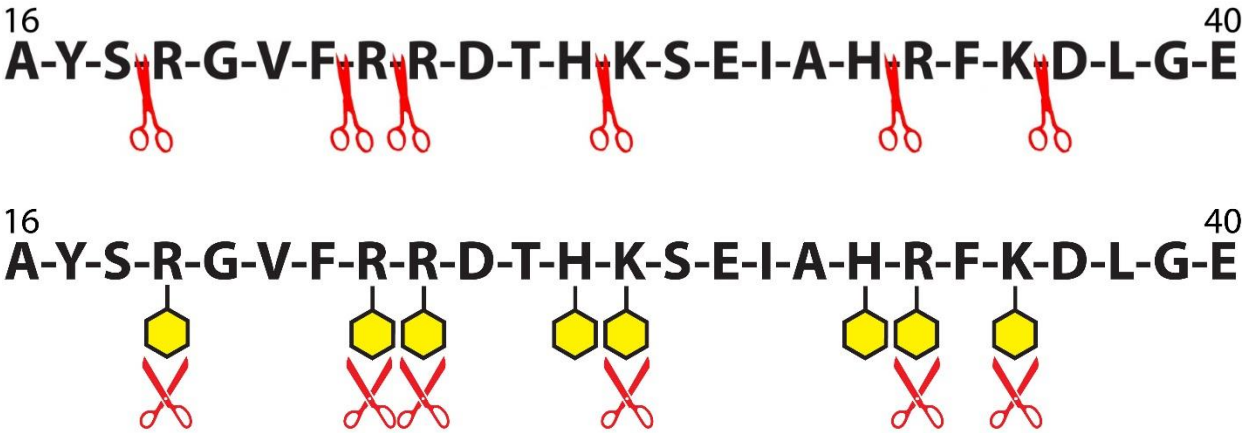


Figure 9.The upper half shows the enzyme trypsin as scissors cleaving the amino acid sequence of the protein. The bottom half shows the covalent binding of colorants to three major amino acids: arginine (R), histidine (H), and lysine (K). The binding of colors to the protein significantly prevents its digestibility by blocking trypsin (open scissors) from digesting the protein, resulting in accumulation of undigested food proteins in the digestive tract.

Other chemicals are able to produce reactions similar to those produced by artificial food colorants. In this heavily industrialized world, chemicals are found not only in the air, water, soil and food, but also in human beings, including in human breast milk. These chemicals include formaldehyde, toluene diisocyanate, trimellitic anhydride, phthalic anhydride, benzene-ring containing compounds, and solvents. When these chemicals or their metabolites bind to human tissues, it results in increasing the body burden of chemicals. This is different from chemical levels as measured in blood or urine, which give a level but not the total body burden, an important factor as many chemicals are stored and not excreted. The immune system will mount an attack on these chemicals bound to human tissues, resulting in autoimmunity.[4] Because antibodies appear in the blood years before the onset of an autoimmune disease, the clinician has the opportunity to greatly help his patients by knowing ahead of time if they are at risk for developing autoimmunity. By guiding the patient with avoidance, prevention and lifestyle changes, autoimmunity may be avoided.[4] These and other chemicals make up the testing in Cyrex Array 11 – Multiple Chemical Immune Reactivity Screen™.

Lectins/Agglutinins. Dietary lectins and agglutinins can also cause similar or even stronger reactions than chemical haptens due to their structure and size. As they are contained in many plants, including cereal grains, legumes and vegetables, and as they are a large part of our diet, they can affect our health. Lectins and agglutinins are carbohydrate-binding proteins present in many plants, and are part of the plant survival mechanism against insects, molds, fungi and diseases. To fully digest lectins, humans need proper and sufficient enzymes. When lectins are consumed by an individual with insufficient digestive enzymes, in addition to maldigestion and nutritional deficiencies, it can contribute to intestinal damage. Lectins can bind to gut bacteria or to gut epithelial cells, or both, causing inflammation and opening of the tight junctions, leading to a leaky gut which is the gateway to autoimmunity.[42 43 44 45 46] Dietary lectins, by binding to gut microbiota, can induce the release of endotoxins such as lipopolysaccharides (LPS), which

increase gut permeability and allow the passage of lectins, food antigens, and bacterial toxins into the circulation. This can result in the binding of lectins to a number of target tissues, including connective tissue, thyroid, liver, pancreas, cardiac muscle, prostate, breast, and brain, and other tissues shown in **Table 2**. Furthermore, activation of the immune system can give rise to antibodies against the lectins, other food antigens, and bacterial toxins due to cross-reaction between different food and bacterial antigens with human tissue. The attack against lectin-bound tissue antigens or tissue antigens may come about as a direct attack or as a result of molecular mimicry, in which the amino acid sequence is similar, and either one can result in autoimmunity[47] (**Figure 10**).

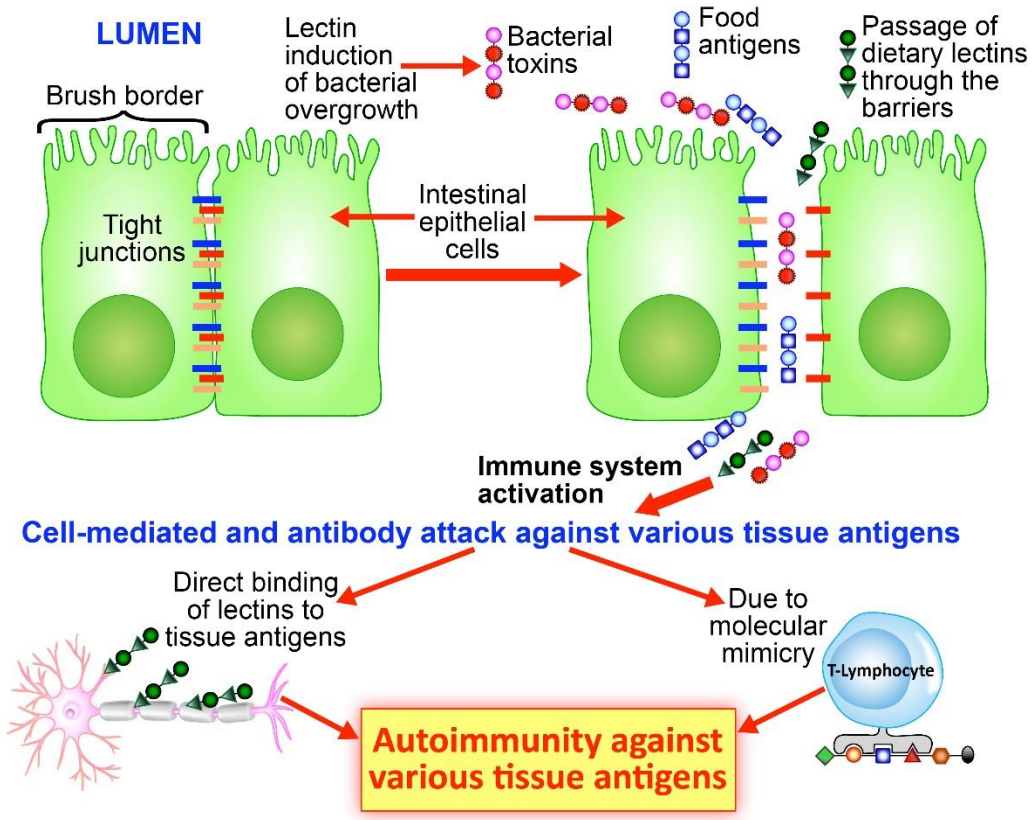


Figure 10. Dietary lectins, their interaction with the gut and immune system, and their contribution to inflammation and autoimmunity. Lectins that infiltrate the body may induce cell-mediated and antibody responses against human tissues due to lectin-binding to tissue and/or molecular mimicry.

Lectins/Agglutinins with Affinity to Specific Tissues

Table 2. Lectins/Agglutinins with Affinity to Specific Tissues [52] (color indicates affinity)

Tissue	Wheat Germ Agglutinin*	Soybean Agglutinin #	Peanut Agglutinin #	Lentil Lectin #	Pea Lectin #	Bean Agglutinins # @
Skin						
Nasopharyngeal epithelium						
Buccal mucosa						
Stomach						
Parietal cells						
Intestinal brush border						
Colonic mucosa						
Connective tissue						
Thyroid						
Cartilage						
Liver						
Pancreas						
Kidney						
Prostate						
Skeletal muscle						
Cardiac muscle						
Breast						
Pituitary						
Eye						
Brain (myelin)						

**Assessed on Array 3 – Wheat/Gluten Proteome Reactivity and Autoimmunity*
 # Assessed on Array 10 – Multiple Food Immune Reactivity Screen
@ Assessed on Array 10-90 – Multiple Food Immune Reactivity Screen

Lectin-induced diseases beyond the gut. In **Table 2** we show lectins and agglutinins that have the potential to bind to a number of tissues throughout the body. For example, lectins bind to islet cells of the pancreas, which can lead to autoimmunity against the islet cells, resulting in type 1 diabetes. Lectins can also bind to glucosaminoglycans and proteoglycans, major components of joints, possibly leading to rheumatic conditions, another autoimmune disorder. Lectin injected into mice caused the lectin to bind to IgG and form rheumatoid factor, inducing rheumatoid arthritis. Lectins can bind to glomerular basement membrane, with the resulting autoimmune response causing glomerulonephritis. Furthermore, lectins may bind to human endometrium, spermatozoa and ova; the resulting autoimmune reaction may cause infertility in men or women.[48 49 50 51 52] **Figure 11** shows the contribution of lectins in the development of various disorders.

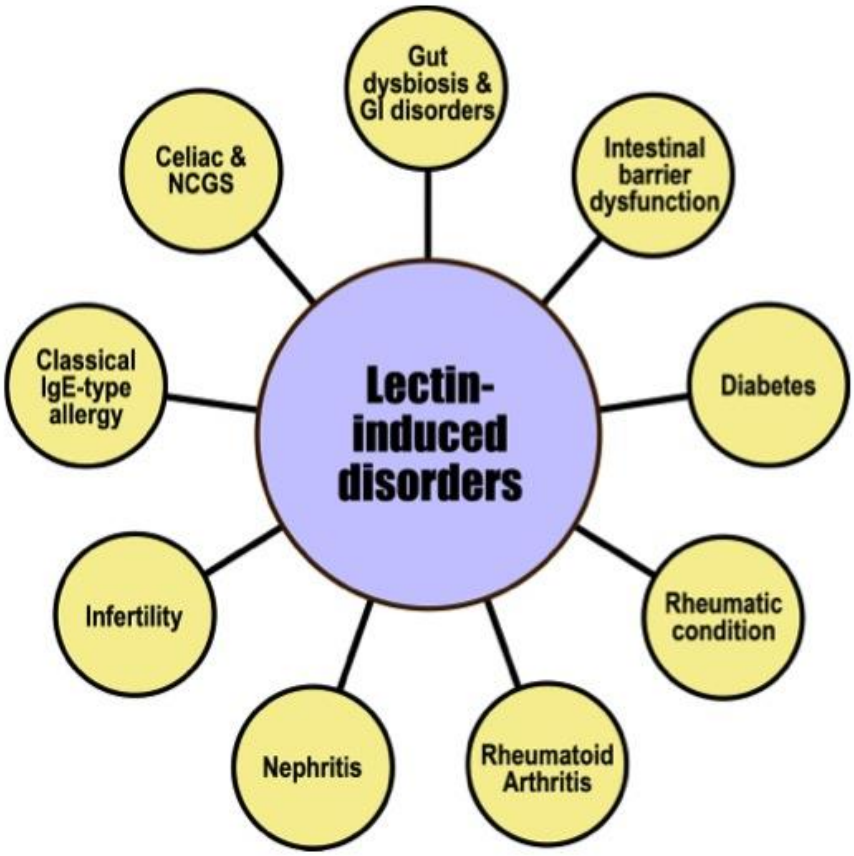


Figure 11. Contribution of lectins in the development of various disorders. Systemic lectins have been shown to contribute to various disorders.

Detection of IgG or IgA antibodies against specific lectins may serve as a guide to clinicians for the elimination of lectins for their patient's diet. A cellular and/or antibody attack against lectin-bound tissue antigens or tissue antigens that share an amino acid similarity with food and bacterial antigens can significantly contribute to the development of autoimmune reactivity and autoimmune disease via molecular mimicry.[47 50 51 53]

Molecular mimicry and cross-reactivity between food antigen and human tissue as the major cause of autoimmunity

Wheat. There are numerous publications regarding foods and immune and autoimmune reactions.[54-86] The two most prominent subjects of these publications are wheat and milk, more specifically gluten and casein. Gluten, for example, is known to be linked to celiac disease (CD) and non-celiac gluten sensitivity (NCGS). Patients with CD have an immune system that may react to a wide range of wheat peptides. NCGS patients and those with Crohn's disease react to a repertoire of wheat antigens, producing IgG and IgA antibodies against them. Continued and unrecognized exposure to wheat brings about a worsening of NCGS and CD and, when left untreated, can lead to autoimmunity. Therefore, those patients with type 1 diabetes mellitus or autoimmune thyroid disease should be tested for CD and NCGS as a significant percentage of patients with one will have the other. This is also true of multiple endocrine disorder,[87] Addison's disease, alopecia,[88] and hypophysitis.[89] In women with unexplained infertility, CD has been found in 4-8%, and successful pregnancy has occurred once diagnosis and appropriate dietary measures were implemented. The infertility also applies to gonadal function in men with infertility. As to osteopenia and osteoporosis, studies have shown that most patients with CD have circulating antibodies to wheat proteins that react against bone structure.[90] Cyrex developed Array 3 – Wheat/Gluten Proteome Reactivity & Autoimmunity™ to assess wheat/gluten reactivity with great sensitivity and specificity. Please refer to the Array 3 Clinical Application Guide for more information. **Figure 12** shows the spectrum of autoimmunity and its association with only one food item: wheat.

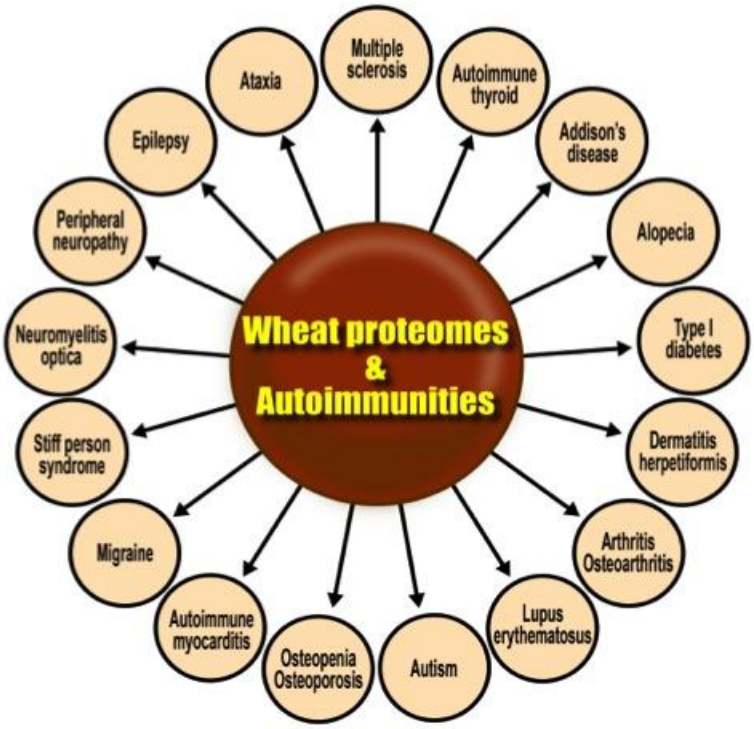


Figure 12. Spectrum of autoimmune disorders associated with wheat proteomes. Wheat/gluten immune reactivity has been linked to various disorders affecting a variety of tissues.

Milk. One of the most common foods to cause immune reactivity is cow's milk, affecting infants, children, and adults. The principle antigenic components of cow's milk are alpha, beta, and kappa casein, butyrophilin and beta-lactoglobulin. Drinking cow's milk early in life may be a risk factor for the development of autoimmune diseases such as celiac disease, Crohn's disease, Behçet's disease, multiple sclerosis, systemic lupus erythematosus, uveitis, and type 1 diabetes in susceptible patients. Research has shown there is significantly higher levels of IgG and IgA antibodies in these disease sufferers compared to normal controls (**Figure 13**).[91 92 93 94]

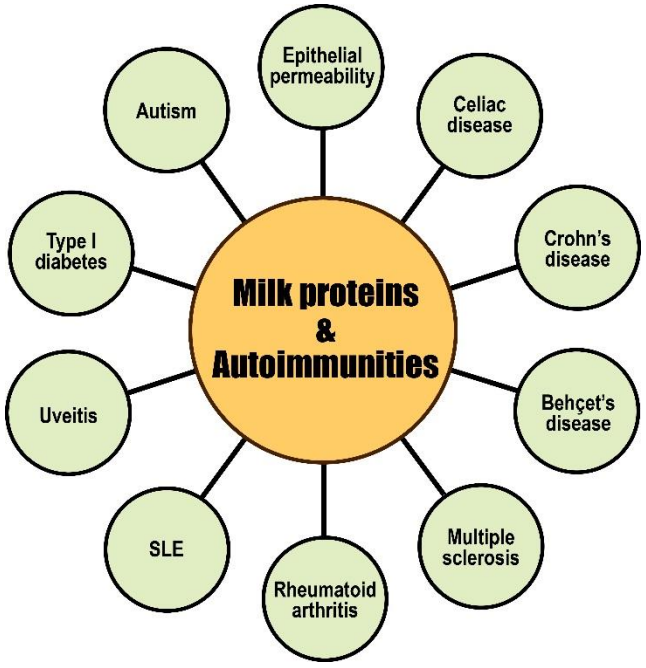


Figure 13. Spectrum of autoimmune disorders associated with milk proteins. Milk immune reactivity has been linked to a variety of disorders affecting various tissues.

One possible mechanism of action in the development of type 1 diabetes with the consumption of cow's milk is the similarity of cow's milk protein to islets of Langerhans cell proteins; this is known as molecular mimicry (**Figure 14**). Cyrex's Array 4 – Gluten-Associated Cross-Reactive Foods & Food Sensitivity™ assesses immune reactivity to milk with six different milk product antigens.

Figure 14. Antigenic similarity between cow's milk protein and beta cell components. Amino acid sequence similarities occur between cow's milk and islet cells, which explain why

cross-reactive antibodies are produced in some patients. Refer to Array 6 – Diabetes Autoimmune Reactivity Screen™ for more information.

Another example of molecular mimicry is alpha-S 2-casein causing uveitis via its sequence similarity to retinal S-antigen (**see Figure 15**). When retinal S antigen was injected into experimental rats, 85% developed uveitis; to demonstrate the role of cross-reactivity in autoimmunity, when alpha-casein was injected, and 50% developed uveitis, a highly significant finding.[95]

Figure 15. Antigenic similarity between αs2-casein and retinal S-antigen. The strong amino acid sequence similarity or molecular mimicry between cow's milk casein, retinal S-antigen and rota virus can lead to cross-reactivity and autoimmune disorders.

Cow's milk may also be one of the causes of multiple sclerosis (MS). Multiple retrospective population based studies in the United States, European countries, Japan, Australia and South Africa, show a correlation of MS with cow's milk consumption. In looking for a mechanism for cow's milk possibly causing MS, there was a high sequence of similarity between a major protein of milk fat globule membrane called butyrophilin (BTN) and myelin oligodendrocyte glycoprotein (MOG), a known autoantigen associated with MS; this again shows molecular mimicry, as depicted in **Figure 16**, where 50% of the amino acids of milk butyrophilin were similar to MOG.[90]

Figure 16. Antigenic similarity between butyrophilin (BTN) and myelin oligodencrocyte glycoprotein (MOG). Amino acid sequence similarities occur between cow's milk butyrophilin and neuronal myelin oligodendrocyte glycoprotein, which explains the cross-reactive antibodies produced in some patients. Refer to Array 7 and 7X – Neurological Autoimmune Reactivity Screen™/Expanded™ for more information on neuronal autoimmunity.

Additionally, small nuclear ribonucleoprotein (SmD1) is an important autoantigen found in patients with systemic lupus erythematosus. Seventy percent of patients with lupus react with this autoantigen. Casein, the protein in cow's milk, cross-reacts with SmD1 and therefore can be a cofactor in lupus.[94]

In a ground-breaking study of 400 healthy donor blood samples for antibodies to specific wheat and cow's milk protein and peptides, as well as to select neurological tissues, Vojdani *et al*. showed that

cerebellar, myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein peptides known for their importance as autoantigens in gluten ataxia and MS.[5] These neuronal antigens can cross-react with wheat and cow's milk protein.[5] The authors state, "The demonstration of molecular mimicry between α-gliadin, cerebellar peptide, milk butyrophilin, and MOG, and the simultaneous detection of antibodies against these proteins even in a small percentage of the general population may have broader implications in the induction of neuroimmune disorders."[5] When the intestinal barrier is breached, these food proteins and peptides can stimulate antigen-specific immune responses both locally in the gut as well as in the periphery. In the majority of the population the cerebellar and MOG normally remains sequestered behind the blood brain barrier (BBB) and therefore do not stimulate neuroautoimmune reactivity. However, nervous system inflammation and BBB breakdown can allow the entry of these cross-reactive antibodies, resulting in neuroimmune disorders.[96 97]

Food Aquaporins. Aquaporins, also known as 'water channels,' are integral membrane proteins that conduct water molecules in and out of cells in the human body. Aquaporins from food sources are highly stable in food preparation and therefore may reach the gastrointestinal as intact proteins or peptides.[98 99] In cases of breakdown in immunological tolerance, aquaporins from foods may become antigenic, and the immune reaction against them could result in antibody production. Aquaporins from some food sources show similarity with human aquaporin (**Figure 17**).[100] The food sources of AQP4 that have been shown to cross-react with human AQP4 include soy, corn, spinach and tomato.[100]

Figure 17. Similarity between human aquaporin-4 and different plant AQP-4. Amino acid sequence similarities occur between human aquaporin and plant aquaporins from soy, corn, spinach and tomato, which explains the cross-reactive antibodies produced in some patients. Refer to Cyrex's Array 20 – Blood Brain Barrier Permeability Screen for more information on assessing the integrity of the blood-brain barrier.

Aquaporin also cross-reacts with legume serine proteinase inhibitors (serpins) found in beans, lentils, peas, peanuts, lupin, alfalfa, and clover.[100] This similarity is shown in **Figure 18**:

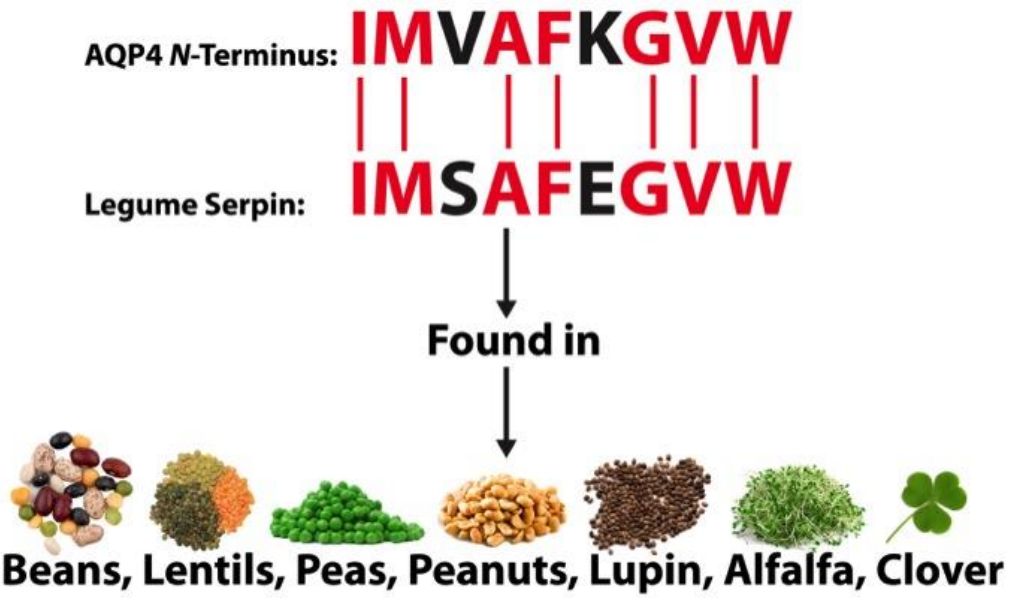


Figure 18. Cross-reaction between *N*-terminus of aquaporin-4 with various legume serine proteinase inhibitors (serpins). Please refer to Array 20 – Blood Brain Barrier Permeability Screen™ for more information on assessing autoimmunity to blood-brain barrier.

These figures show aquaporin and serpin cross-reacting with foods, which may cause immune reactivity and the formation of cross-reactive antibodies. If these antibodies cross the blood-brain barrier (BBB) in susceptible individuals, it can result in neuromyelitis optica (NMO), a form of MS (**Figure 19**). These are severe neuroautoimmune disorders that affect the gray and white matter in the brain and spinal cord, causing demyelination, axonal damage and necrosis, ultimately resulting in paralysis and sensory loss.[101] Seventy-five percent of NMO cases are associated with IgG1 antibody development that binds selectively to aquaporin-4 (AQP4),[102] [103] which is expressed in the astrocytic foot processes around the BBB.[104]

This can be of clinical importance for adjusting the diet in patients with demyelinating disorders such as MS and NMO and/or those with a family history of such disorders

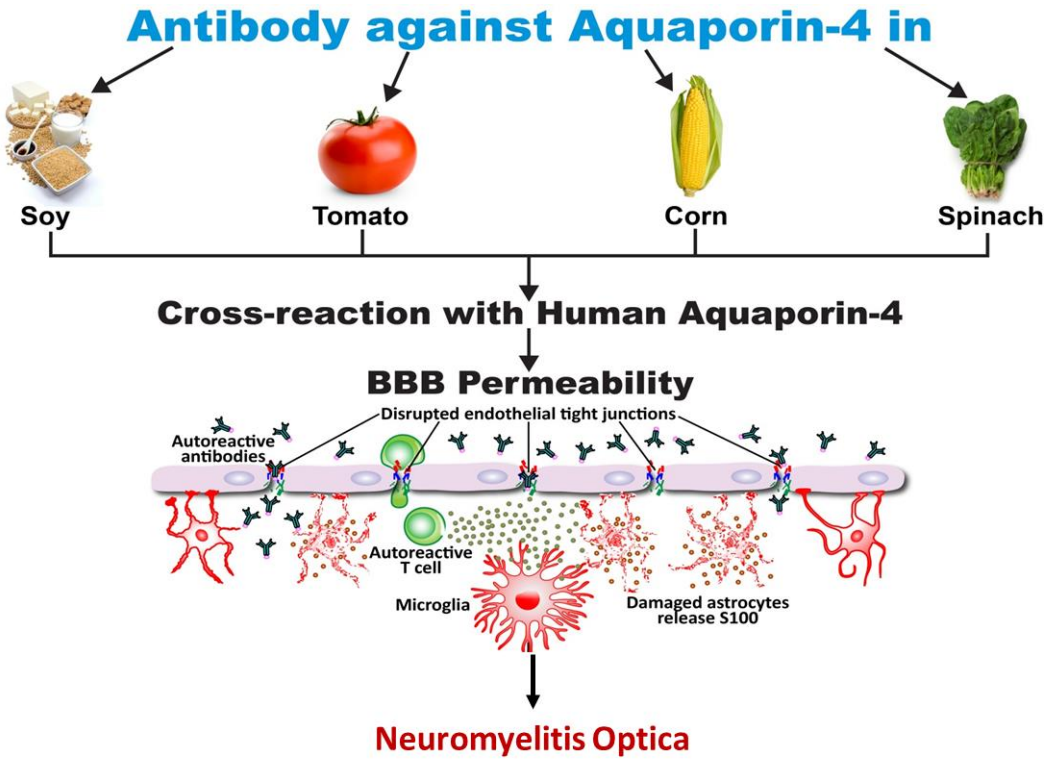


Figure 19. How plant aquaporin-4 can lead to neuromyelitis optica. Antibodies against plant aquaporins can trigger a cascade of immune reactivity that can lead to neuromyelitis optica. Please note, Array 10-90 assesses immune reactivity to Spinach Aquaporin, while Array 10 assesses immune reactivity to Corn, Soy, Spinach and Tomato aquaporins.

Glucans. Glucans are produced by fungi, yeasts, grains and seaweed. They are the constituents of the cell wall of certain pathogenic bacteria (*Pneumocystis carinii, Cryptococcus neoformans*) and fungi (*Aspergillus fumigatus, Histoplasma capsulatum, Candida albicans, Saccharomyces cerevisiae*). When SKG mice, which develop spontaneous IL-17–dependent autoimmune inflammatory arthritis under conventional microbial conditions, initiated by pulmonary fungal infection,[105] were injected with beta-glucan, researchers saw that an interaction between innate control of microbial immunity and autoimmunity underlies the tissue-specificity of the initiation of arthritis and spondylitis.[106] Yoshitomi and colleagues administered beta-glucan to SKG mice and concluded, "environmental agents, such as fungi and viruses, may evoke autoimmune arthritis similar to rheumatoid arthritis." Individuals who harbor arthritogenic T cells and are exposed to beta-glucans may activate antigen presenting cells in an antigen non-specific manner, and thereby activate preexisting arthritogenic T cells.[107] The end result may be arthritis. In fact, elevated levels of antibodies against glucans were found in patients with rheumatoid arthritis and systemic lupus erythematosus.[108]

Another glucan-related antigen is found in *Saccharomcyces cerevisiae*, a yeast commonly used in the food industry. Antibodies to *Saccharomyces cerevisiae* (ASCA) are a serological marker for Crohn's disease and are highly predictive of inflammatory bowel disease and Behcet's disease, spondyloarthritis, celiac disease, intestinal tuberculosis, primary biliary cirrhosis, autoimmune hepatitis, type 1 diabetes and autoimmune thyroid disease. A recent study shows that ASCA are also found in patients with systemic lupus erythematosus.[109]

Fruit pectins. Fruits, such as apple, berries, grapefruit, orange and quince, contain pectin. Pectins may play a role in the pathogenesis of joint disorders. Traditionally, when suspecting rheumatoid arthritis (RA) in a patient, a rheumatoid factor test is ordered, along with anti-cyclic citrullinated peptide (anti-CCP) antibodies and a sed rate. This does not take into consideration the possible causes for RA. A recent study shows that testing for polygalacturonic acid (PGA) antibodies, a major component of food pectin, strongly correlates with RA[110] (**Figure 20**).

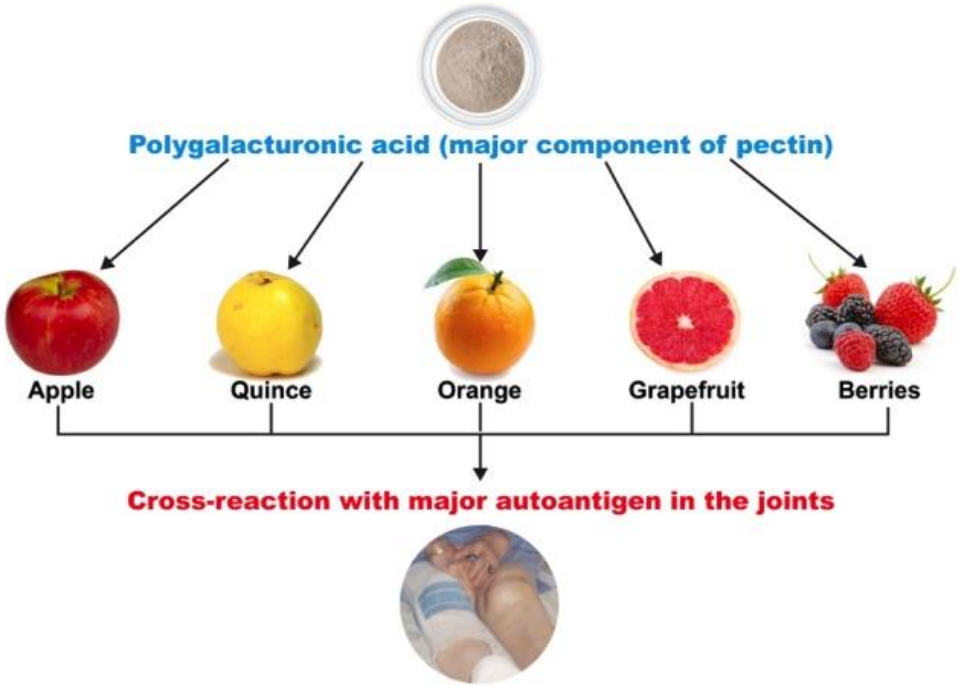


Figure 20. Cross-reaction between food pectins and autoantigens in joints.

This demonstrates that by removing the triggers found in foods one may significantly alter the progression of RA.

Glycine-rich food proteins. Glycine-rich food proteins are found in gelatin, meat, soy protein, chicken, egg, seeds, cereals, fruits, vegetables, French beans, and rice. They can contribute to autoimmunity by cross-reacting with collagen, keratin, and actin, and ribonuclear protein as shown in **Figure 21**.

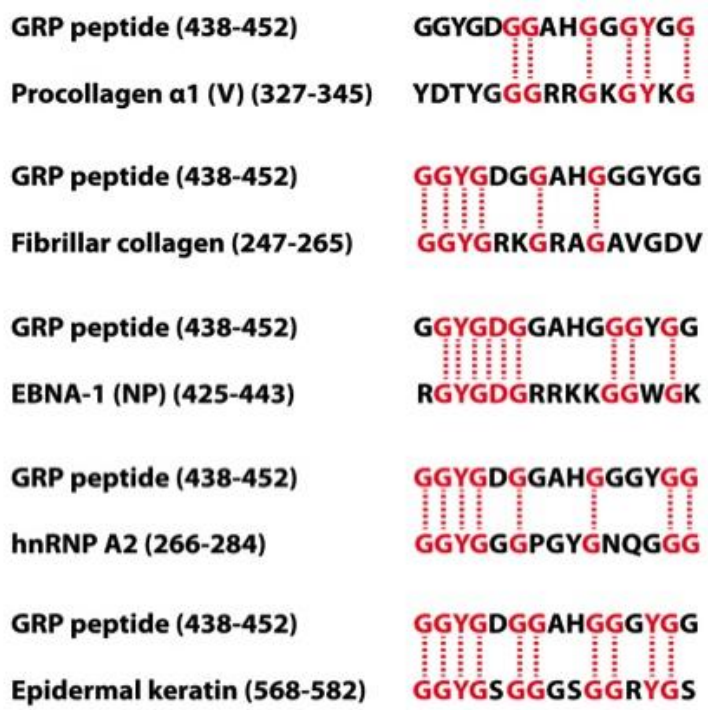


Figure 21. Degree of homology between glycine-rich proteins (GRP) and various antigens.

Autoimmune disorders that have shown the involvement of glycine-rich food proteins include RA, systemic lupus erythematosus, mixed connective tissue disease, MS, and type 1 diabetes. Epitopes in plants and humans may be responsible for an autoimmune response in susceptible individuals. It may also indicate that the antigen-spreading of a particular sequence among divergent proteins may participate to initiate or amplify an immune response. The finding of a common peptide epitope able to elicit an immune response in patients with food allergy and different autoimmune disorders gives rise to the question of a possible link between food antigens, gut mucosa and systemic immune response.[111]

Food proteins reacting with purified blood components of lupus patients. Antibodies that react with the small nuclear ribonucleoprotein particles were detected in the sera of patients with lupus. These anti-Sm autoantibodies of lupus patient's sera (**Figure 22**) also cross-react with common food proteins such as soybean, corn, spinach, and carrot. This cross-reactivity to various food antigens was shown by applying affinity purified anti-Sm antibodies from patients with lupus to different food antigens. As demonstrated in **Figure 22**, these anti-Sm antibodies reacted strongly with soybean, spinach, corn, and carrot. This may imply that these food proteins play a role in the production of anti-Sm antibodies and hence in the etiology of lupus.[112]

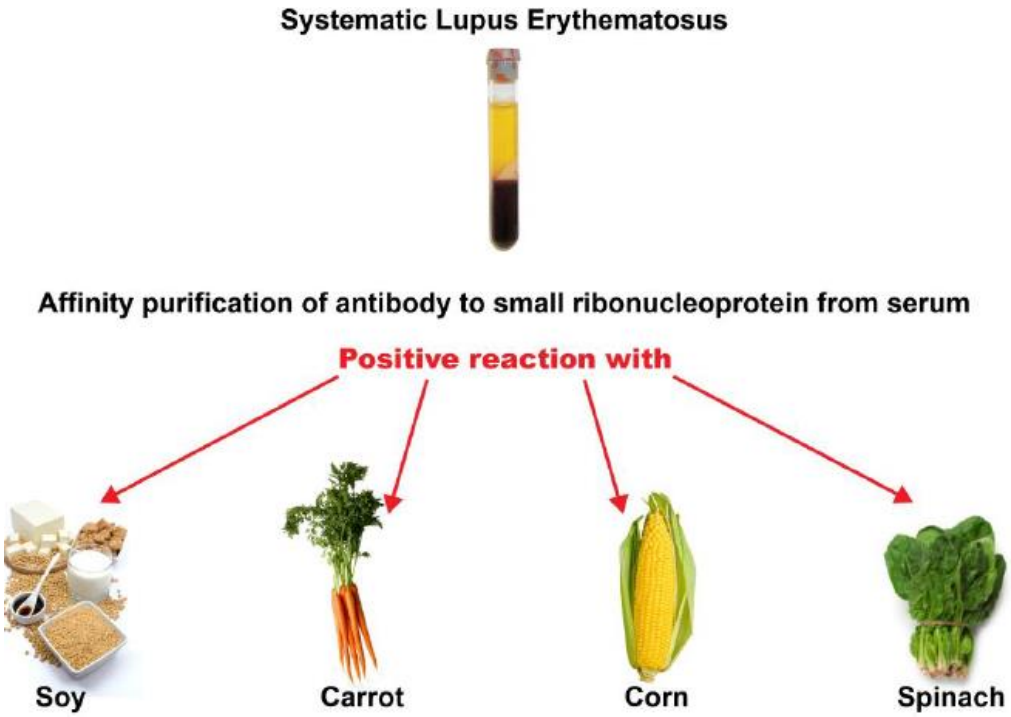


Figure 22. Cross-reaction between lupus antigen and different plant proteins. Anti-Sm antibodies reacted strongly with soybean, spinach, corn, and carrot. This may imply that these food proteins play a role in the production of anti-Sm antibodies and hence in the etiology of lupus.

Food proteins reacting with purified blood components of scleroderma patients. In another similar study in patients suffering from scleroderma, purified Scl-70 sera cross-reacted with plant DNA topoisomerase (**Figure 23**). Plant topoisomerase are enzymes found in wheat germ, peas, corn, and spinach. Similarly to the lupus patients' sera mentioned in the previous section, the sera from scleroderma patients were purified to homogeneity using Scl-70. This purified sera from scleroderma patients cross-reacted with these four foods. The inflammation of the esophagus in scleroderma patients may have dietary correlation related to this organ's exposure to the antigens present in these four foods. With this knowledge, clinicians can help their patients with a diet of avoidance that can have preventive measures.[113]

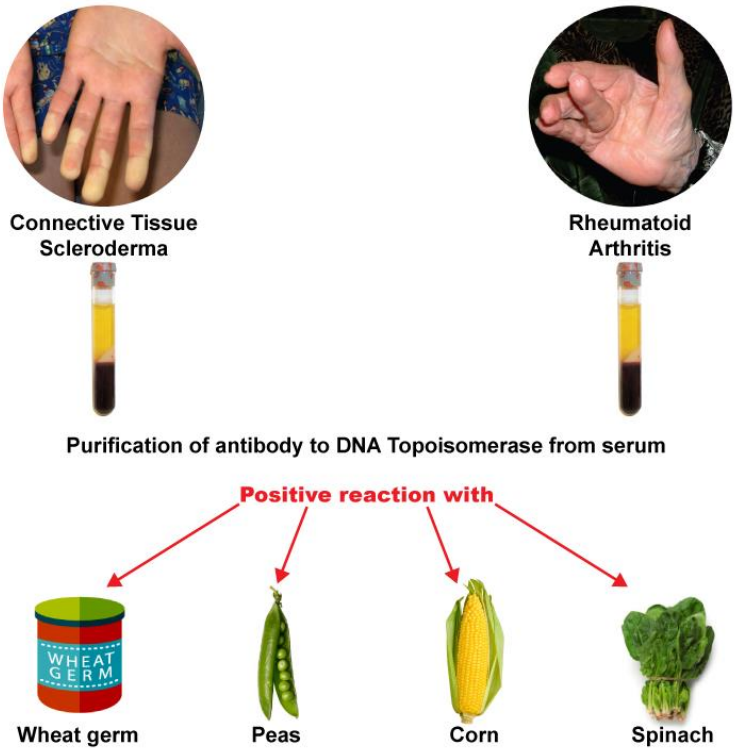


Figure 23. Cross-reaction between human and plant topoisomerase.

Shrimp tropomyosin. Shrimp tropomyosin is a cytoskeletal microfilamental protein that regulates actin mechanics. Tropomyosin is a common antigen in fish and shellfish.[114] Tilapia tropomyosin (TM) showed 53.5% homology to TM from shrimp and (87.7%) to human TM isoform 5.[115] Although the function of tropomyosin in muscle has been well characterized, its function in epithelial cells, is unclear.[116] Autoantibodies against human TM have been implicated as a causative agent in inflammatory bowel disorders.[116] This may be due to cross-reactivity of dietary tropomyosins with human epithelial tropomyosin (**Figure 24**).

Figure 24. Cross-reaction between shrimp and human tropomyosin. Refer to Cyrex's Array 5 – Multiple Autoimmune Reactivity Screen for assessing autoimmune reactivity to human tissue tropomyosin.

Gums. Most food-related gums are composed of complex and variable mixtures of oligosaccharides, polysaccharides and glycoproteins with an extremely high molecular weight polysaccharide attached to a hydroxyproline-rich polypeptide backbone.[117] Gums have wide industrial uses, including:

- Food industry - stabilizers, thickening agents, gelling agents, emulsifiers, fixing agents in foods and soft drinks
- Cosmetics - stabilizers, thickening agents, gelling agents
- Manufacturing – printing, textile, pottery, lithography.

Because of their high molecular weight (200-2,000 kDa),[117] if the partially digested molecules of certain gums manage to get into the circulation, they would induce a very strong immune response that would result in high levels of IgG and/or IgA antibodies against the gum molecules. In a study on gum reactivities, 288 healthy subjects were evaluated for IgE and IgG immune reactivity to a set of gums used in the food industry.[118] The gums are listed in order from most to least IgG reactive – carrageenan, mastic gum, locust bean gum, xantham gum, β-glucan, gum tragacanth, guar gum.[118] Results of this study indicates that a significant percentage of the healthy population is not only exposed to various gum products, but immunologically reacts against them.[118] Cross-reactive carbohydrate determinants (CCD) containing fucose and xylose exist in almost all plant extracts and thus there can be homology between repetitive polysaccharide sequences of gums with plant enzymes such as horse radish peroxidase or bromelain, pollens, trees, celery, potato, tomato, beans and pea (**Figure 25**).[119 120 121 122] This finding gives substance to the concept that individuals may produce antibodies against the CCD of gums which then cross-react with many other foods and other environmental antigens.[118] Therefore, immune reaction to gums may play a role in autoimmune reactivity.

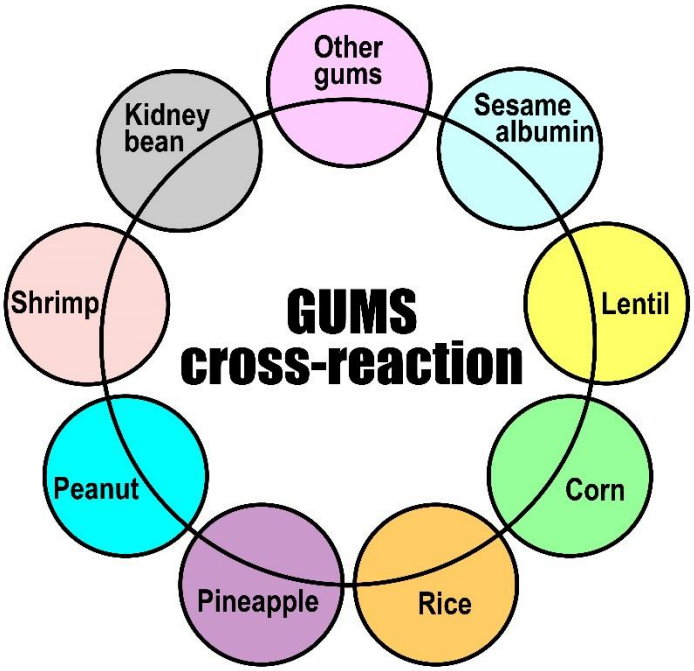


Figure 25. Cross-reactivity of gums with various food antigens. Gums have been shown to cross-react with a variety of food proteins.

Oleosins. Oleosins are antigens within the oil of seed plants with high oil content. An oleosin is a structural protein found in the monolayer of oil bodies.[123] [124] Many of the nut oils available at the local grocer undergo minimal processing and therefore may contain residual nut antigens.[125] [126] In other words, they may be contaminated by nut meat or kernel antigens along with the oil antigen known as oleosin. Oils that are made with the highest standards of oil extraction theoretically do not contain meat/kernel proteins; however, they may have oleosins, which have been shown to elicit immune reactivity.[127] [128]

In a Nutshell

Researchers are identifying the pathogenic roles of many food antigens, such as wheat, dairy, lectins, and chemicals. By being aware of the potential damage some food antigens can cause, expertly assessing them in patients and applying tailored dietary protocols, practitioners can realize better clinical outcomes in their patients.

CLINICAL APPLICATION OF FOOD IMMUNE REACTIVITY TESTING

A physician is often faced with a patient who gives a history of unexplained, non-specific, chronic symptoms and complaints, such as chronic fatigue, malaise, muscle and joint aches and pains, low grade fevers, irritable bowel-like symptoms, sleep disturbance, mental fogginess, blurred vision, unusual headaches, dizzy spells, short-terms memory loss, and cognitive function

problems. Typically, these patients have previously sought the help of other physicians, who have run the usual routine tests with mostly normal findings, and tried various symptomatic treatment modalities, all to no avail. The cause of these symptoms may be an immune reaction to food components, or to the chemical additives in food, or to chemicals bound to food antigens. Testing for possible immune reactions to foods can be of importance in dietary management of patients especially for those with known autoimmune conditions. However, current laboratory testing for food reactions lack both specificity and sensitivity.

This begs the question: how many patients are there who have been tested for food immune reactions and the laboratory reported false/positive or false/negative results?

Clinical Scenarios

Please watch the Array 10 Interpretation Webinar, available on-line, for detailed case presentations on multiple food immune reactivities.

Cyrex Helps Connect the Dots Between Food Immune Reactivity and Autoimmunity

From this we learn that food immune reactions and molecular mimicry may cause autoimmune disorders and diseases. We are addressing a much larger issue: the IgG and IgA immune reactions to food proteins and the chemicals in foods potentially causing disorders that already affect over 53 million Americans. In other words, we are testing for what may be the triggers of the 80 known autoimmune diseases and associated disorders.

The extensive literature research, presented in the previously mentioned references above, found that there is an association between food immune reactivity and 24 major autoimmune disorders, including rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus among others. (See **Figure 26**).

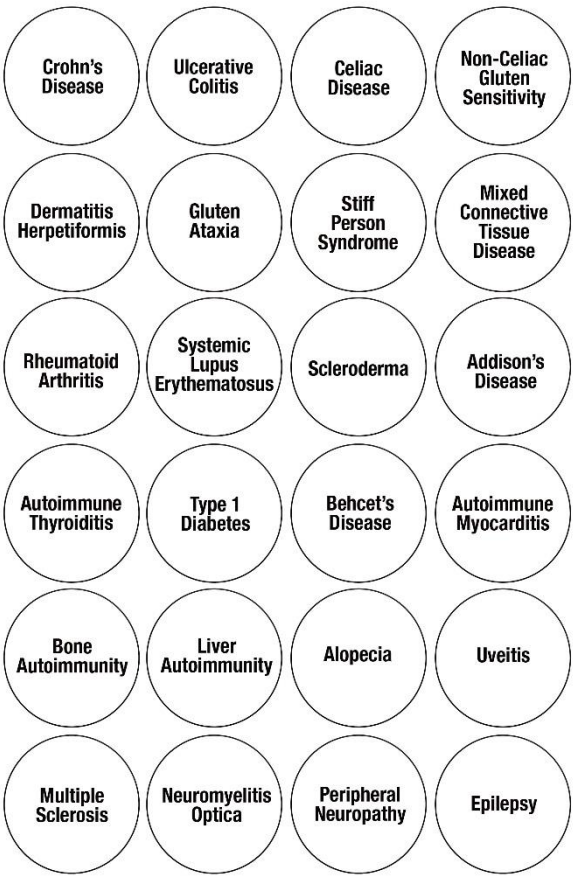


Figure 26. Various autoimmune disorders associated with food immune reactivity. Please refer to Array 5 – Multiple Autoimmune Reactivity Screen™ to assess autoimmune reactivity to self-tissue.

This is why it is so important to test patients for food immune reactivities based on reliable laboratory methodology in an attempt to prevent years of suffering in patients who may unknowingly be in the process of developing an autoimmune disease. Unless these 10 points below are applied to laboratory testing, reliability will be lost and patients will not be helped.

1. **Testing both Raw and Cooked** forms of common foods, as heating food above 118˚ Fahrenheit changes its protein structure and therefore its antigenicity.
2. **Testing Cross-Reactive Pan-Antigen Isolates**, which are antigens known to cross-react with human tissues and can result in tissue damage.
3. **Testing Multiple Food Protein Interactions**, as food protein interactions can change their antigenicity.
4. **Testing Large Gum Molecules**. Gum reactivity can be a serious problem especially for people on a gluten-free diet. Gluten-free products often use gums as a substitute for gluten to hold ingredients together.
5. **Testing Binding Isolates**, as plant derived Agglutinins have an affinity for specific human tissues, which can trigger an autoimmune response.

6. **Testing Tissue-Bound Artificial Food Colors**. Artificial food colors are small molecule chemicals. The right way to measure patients' reactivity, is to assess levels of antibodies to chemicals bound to human tissue.
7. **Testing Amplified Antigenic Proteins and Peptides**. Cyrex Targeted Protein Amplification Process detects the possible reactivity to a much smaller specific peptide within that whole food.
8. **Testing Oleosins**. Oils, once thought to be free of proteins, do indeed contain proteins called oleosins. These proteins can elicit immune reactivity.
9. **Testing Meat Glue**. Or Re-Formed Meat. Meat-glue, a combination of transglutaminase with other ingredients, is used to turn small pieces of meat into larger pieces of meat.
10. **Dual Antibody Detection System**. Some patients produce more IgA than IgG, or vice versa. By combining the two on one panel, Cyrex reduces the possibility of missing immune reactivity.

Array 10-90 can be used to:

- Evaluate immune reactions to foods, raw and/or modified, food enzymes, lectins and artificial food additives, including meat glue, colorings and gums.
- Early detection of dietary-related triggers of autoimmune reactivity.
- Monitor the effectiveness of customized dietary protocol in your patient.

Array 10-90 is recommended for patients who:

- Seek a life-long health and wellness strategy.
- Present with unexplained symptoms whether gastrointestinal, neurological, dermatological or behavioral in nature.
- Are suspected of having increased intestinal permeability, which is the gateway for environmentally-induced autoimmune disorders.

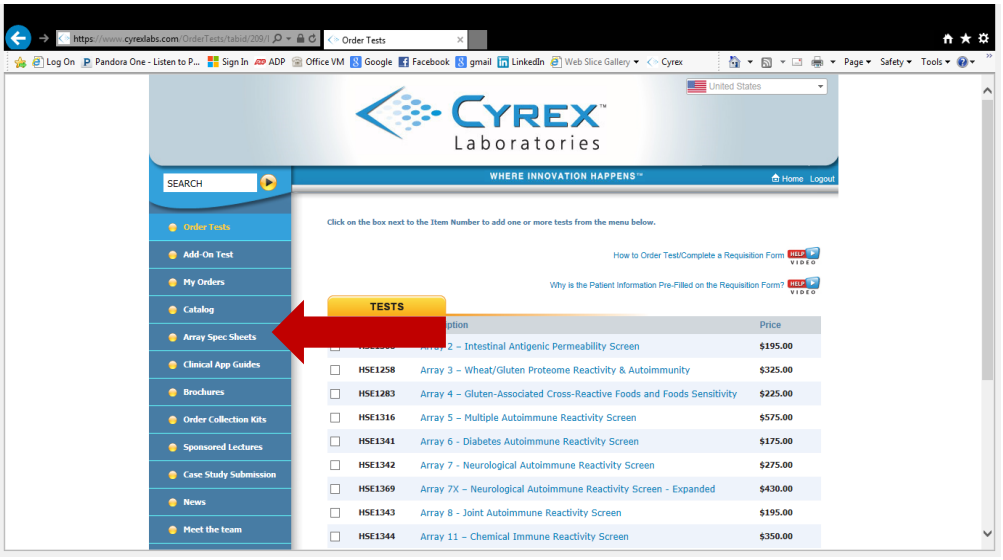
CLINICAL INTERPRETATION FOR ANTIBODY ARRAY 10-90 – MULTIPLE FOOD IMMUNE REACTIVITY SCREEN

Array 10-90 test results are reported as numeric values. If the patient's numeric value falls within the functional normal range, the value is placed in the "In Range/Normal" column. If the value falls within the non-functional normal range, the value is placed in the "Equivocal" column. If the patient has an elevated immune response that falls outside the reference range, the value is placed in the "Out of Range" column.

Functional medicine practitioners often interpret Equivocal results as positives. Interpretation of elevated level of serum antibodies against notable Array 10-90 antigens is shown in the following table.

ANTIGEN	RESULT	CLINICAL NOTES	SUGGESTIONS
Cooked v Raw	If both forms are +	When heating food above 118˚ the proteins can change, making them more, or less, antigenic.	Abstain from all forms
	If raw is the only +		Patient may tolerate cooked forms
	If cooked is the only +		Patient may tolerate raw form
Bean Agglutinins	+	Agglutinins are well known for binding to human tissues, and thus can promote an autoimmune reaction against the tissue.	Abstain from all antigenic agglutinins and follow up with human tissue autoimmune reactivity testing
Spinach Aquaporins	+	Aquaporins from food sources show similarity to human aquaporin, and thus they have high potential for triggering autoimmunity to nervous system tissues.	Abstain from all antigenic aquaporin-containing foods and follow up with blood brain barrier permeability and neurological tissue autoimmune reactivity testing
Food Coloring, artificial	+	This is a measurement of immune reactivity to artificial food coloring bound to human tissue, which indicates body burden and possible autoimmune reactivity.	Abstain from all foods and topical products containing artificial food colorings and follow up with chemical immune reactivity and human tissue autoimmune reactivity tests
Meat Glue	+	Clinicians should be aware that people who consume meats may be consuming meat glue. Most commercial meat glue is derived from bacteria; however some may still be sourced from animal blood. Ingredients are added to the transglutaminase and casein to form the final meat glue product. We combined commercial meat glue with ground beef to make our antigen.	Educate patient on the potential exposures to meat glue and advise abstinence from consuming such products. The patient may be reacting to the meat glue, the cooked beef, or one or more of the individual ingredients used in meat glue. Check the ingredients list on the Specification Sheet. Cross-reference the individual ingredients for positive reactions.
Shrimp Tropomyosin	+	Shrimp tropomyosin is a specific shrimp antigen. When assessed alone it is more sensitive than measuring many shrimp proteins. Cross-reactivity among shrimp tropomyosin and human tropomyosin has been identified.	Abstain from all forms of shellfish and follow up with human tissue autoimmune reactivity testing.
Potato, white, cooked (fried)	+	In order to fry a potato, oil must be used. There are a variety of oils. Cyrex's potato was fried in canola oil.	The patient may be reacting to the potato or the oil. Cross-reference the canola oil for positive reactions.

For details of each antigen on Array 10-90, please refer to the individual food antigen specification sheets.



What can the clinician do to help these patients?

The clinician can detect the possible triggers of their patients' symptoms at the earliest possible stage, remove the offending agent, and repair the damage that these immune reactions may have caused. The current best clinical approach is to implement an individualized diet and lifestyle plan, along with other treatment modalities depending on each patient's health status. Implementation of these modalities gives the gut barriers time to repair themselves. Once this is accomplished, oral tolerance against the offending food is successfully re-established, and an excellent prognosis follows.[5] Leaving food immune reactivities undetected in a patient may result in an autoimmune reaction, and then potentially develop into an autoimmune disease.[3 5 100 129 130 131 132 133 134]

Depending on what food and/or food-related items a patient reacts to, the clinician may want to order additional testing. For example, if the patient reacts to artificial food coloring, a chemical antibody assay and an autoimmune antibody assay will help better determine the patient's health status and implement appropriate treatment. Another example would be the patient who reacts to agglutinin and/or aquaporin. Testing would show food immune reactivity, whether or not the patient is in the process of developing or has developed an autoimmune reaction.

SPECIMEN REQUIREMENT

2 mL serum
Ambient

RELATED TESTING

- **Antibody Array 2 – Intestinal Antigenic Permeability Screen (Serum)**
- **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 – Multiple Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 11 – Chemical Immune Reactivity Screen (Serum)**
- **Antibody Array 20 – Blood Brain Barrier Proteins Screen (Serum)**

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