CLINICAL APPLICATION GUIDE

ARRAY 12

ARRAY 12 – Antibody

Pathogen – Associated Immune Reactivity Screen™

Cyrex™ Laboratories

TABLE OF CONTENTS

CLINICAL APPLICATION GUIDE TO PATHOGEN-ASSOCIATED IMMUNE REACTIVITY SCREEN™

OVERVIEW

In the time-line of life on Earth, humans are late-comers. Many microorganisms predate humans by billions of years,[1 2 3] thus they have had more time to evolve, adapt and thrive in our changing ecosystem. When humans came to dominate the surface of the planet, microorganisms continued to survive undetected. As human populations grew, they began to migrate and domesticate animals, which exposed them to additional microorganisms and at the same time, allowed microorganisms to spread world-wide. This micro-world consists of bacteria, viruses, parasites, fungi, and stealth organisms; tiny beings that can have large consequences in some hosts.

The human body is home to a variety of commensal microbes that help keep the body healthy. In the gastrointestinal tract alone, the microbiota outnumber human cells 10 to 1.[4] Commensal microorganisms regulate some important metabolic and physiological functions of the host, and aid in the maturation of the immune system in early life, contributing to host homeostasis throughout one's lifetime.[5 6 7] Factors that influence the microbiota include the human genetic makeup, diet, environmental exposures, stress and activity.[8] Indeed, new industries, new chemicals and drugs and new foods are challenging the human immune system at an alarming rate. When the human host experiences overwhelming environmental stressors, the microbiome may become imbalanced and in such a state, can contribute to pathogen invasion, acute infection, colonization and, eventually, autoimmunity.[7]

Array 12 – Pathogen-Associated Immune Reactivity Screen™ assesses IgG immune reactivity to pathogens that are documented triggers or exacerbators of autoimmunity. Tick-borne pathogens are obvious triggers of Lyme disease, however there are additional pathogens that haven't been in the limelight. One example is the opportunistic bacterium *Acinetobacter*, which, due to cross-reactivity with neurological tissues, has been shown to play a role in multiple sclerosis. *Giardia lamblia* is well known as a cause of watery diarrhea, but less known for persisting in asymptomatic patients where the pathogen destroys intestinal villa thereby preventing nutrient absorption. *Giardia* can then cross-react with structural proteins such as tubulin and actin, which then triggers autoimmunity of the joints. Array 12 contains a variety of pathogens including oral pathogens, gastrointestinal pathogens, gastrointestinal parasites, bacterial and stealth pathogens, environmental molds, viral pathogens and tick-borne pathogens. Each of these pathogens is described below; additionally, we have written white papers detailing the autoimmune mechanisms of each pathogen.

Acute Versus Chronic Infection

The human immune system is responsible for an enormous duty; to recognize and ignore all the cells and tissues within our body (self), and at the same time, attack any and all invaders, such as foreign cells, dietary proteins and chemicals, as well as viruses, bacteria, toxins, and fungi (non-self). Something in this delicate balance has changed over the last 50 years and it is pushing our immune systems to its limit: we are at the edge of our immune system's capacity. Interference with this balance by environmental triggers can result in over-activity to harmless antigens, leading to autoimmunity (**see Figure 1**).

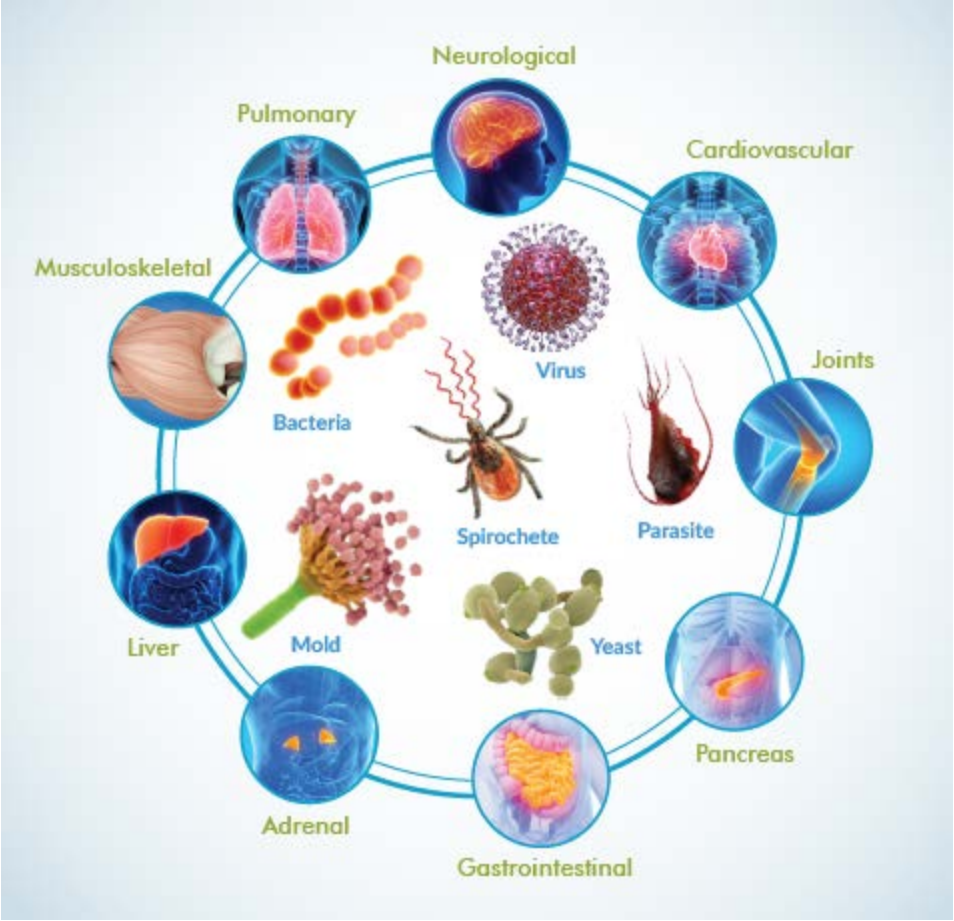

Figure 1. Pathogens have the capacity to induced autoimmune reactivity. *Chronic exposure to a pathogen and/or its biotoxins can contribute to the autoimmune process by upregulating inflammatory immune chemicals or by cross-reactivity between the pathogen and human tissues. Entry of many pathogens is through the gastrointestinal tract, however, once they thrive within the body, they can have far-reaching effects.*

When a pathogen invades the human host, it can cause an acute infection. Most of these acute infections manifest as flu-like symptoms such as diarrhea, fatigue and fever. After the acute phase is complete, it is often thought that the pathogen that ignited the acute response has been cleared by the immune system. In reality, often, after the acute infection, the pathogen remains in human cells where it can thrive in a reactivating or latent state, all the while excreting its biotoxins. To make matters more complicated many people acquire and carry a pathogen without ever experiencing an acute infection.

Although the acute infection stage can be life-threatening in some situations, sometimes it is the chronic, reactivating, latent pathogen exposure that can significantly alter the host's quality of life.

The undetected pathogen may not result in clinical complaints for years or even decades. See **Figure 2** for a review of immune system. After invading ~~in~~ the body, a pathogen may infiltrate tissue cells and there remain safe from immune attack, but still release its toxins into the extracellular medium, thereby affecting distant tissues. Depending on the pathogen type, autoimmunity against human tissues may ensue through different mechanisms including molecular mimicry, polyclonal activation, bystander

activation, epitope spreading, super antigen release, viral persistence, dysregulation of immune homeostasis, and autoinflammatory activation of innate immunity.

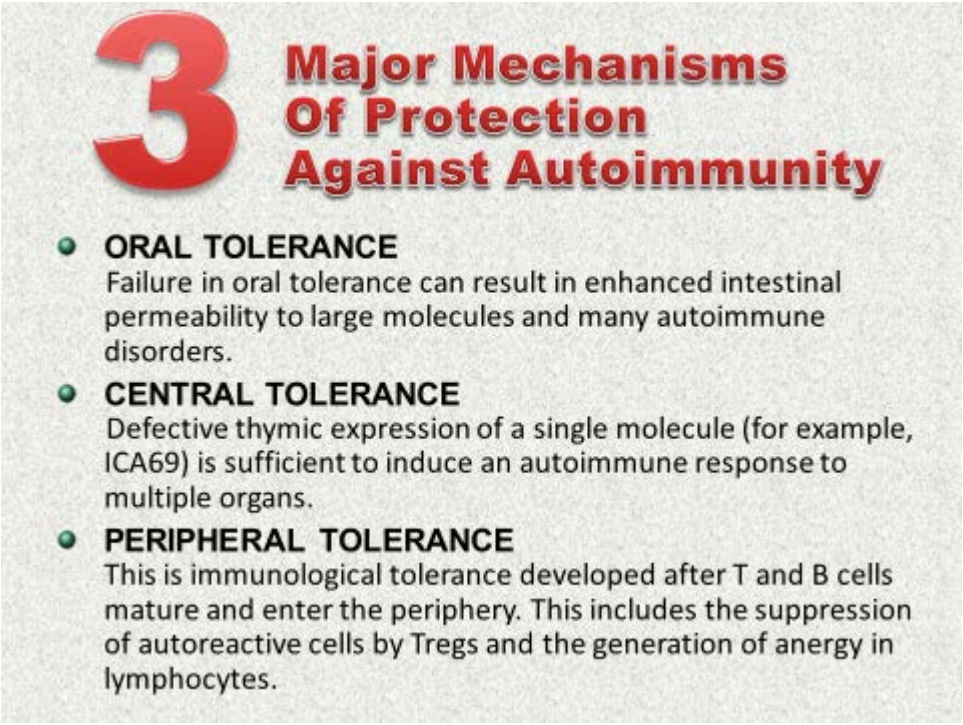

Figure 2. The mechanisms by which the immune system protects the human body. *When tolerance fails the human body, pathogens can remain in the system and contribute to disease pathogenesis.*

The human body has multiple barriers to protect itself from outside invaders. These barriers include gastrointestinal, lung, skin, and urogenital tract. The human weak point and common point of entry for pathogens is the gastrointestinal tract. For a thorough evaluation of the patient adversely affected by pathogenic exposure, via the gastrointestinal tract, as assessed in Array 12, the following outline (**Figure 3**) may guide the clinician in determining the extent of the damage in his/her patient.

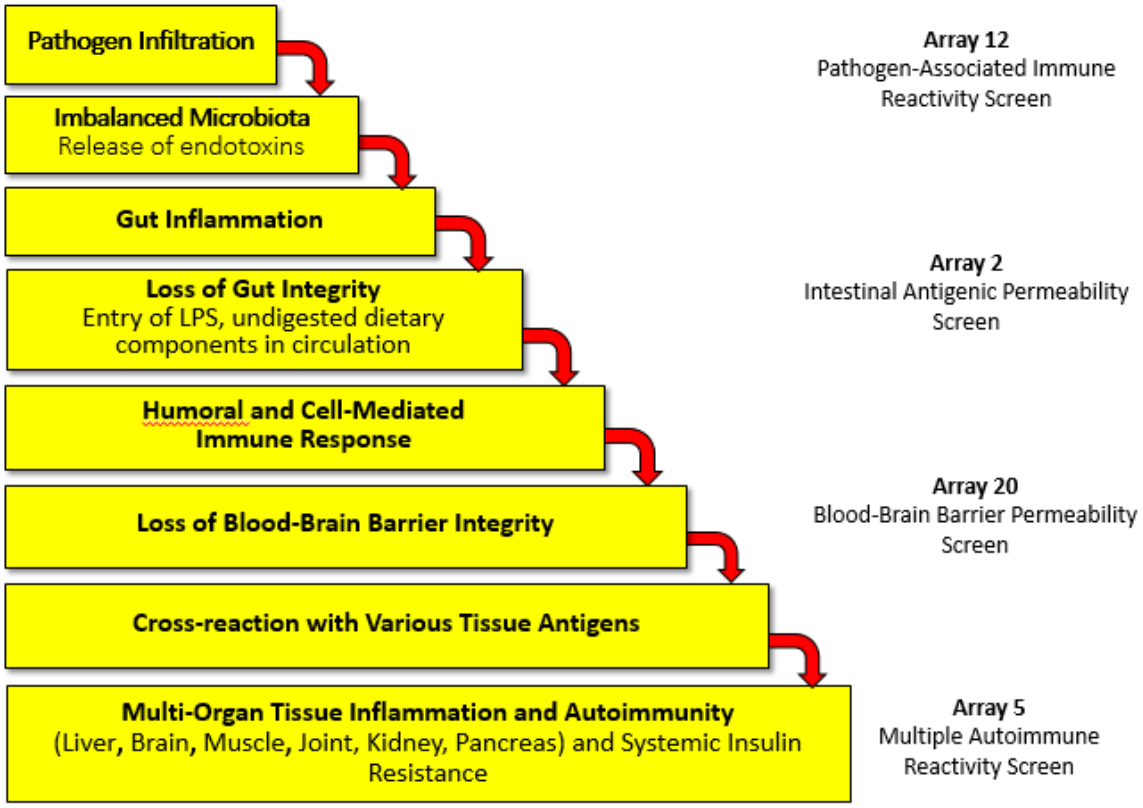

Figure 3. Pathogen invasion cascade. *The cascade of events begins with pathogenic antigens, causing disturbance in gut flora and a sequence that results potentially in autoimmunity. The Cyrex System™ includes laboratory tools that can assist the practitioner in assessing not only presence of persistent pathogen exposure, but also barrier permeability, release of bacterial endotoxins into the bloodstream and autoimmune reactivity to a variety of target tissues.*

Although the precise mechanisms by which pathogens induce autoimmune disorders, are still being researched, it is clear that the breakdown in immunological tolerance and dysfunction of immune homeostasis plus chronic reactivating pathogen exposure are major components in the induction of autoimmune reactivities followed by autoimmune diseases.

In the Microcosm

Although the acute infection stage can be life-threatening in some situations, ~~it is~~ the chronic, reactivating, latent pathogen exposure can also significantly alter the host's quality of life.

The Cyrex Difference

Acute infections can be assessed by measuring IgM antibodies specific to the pathogen, or by culturing stool or saliva samples. When the acute infection phase is complete, IgM antibodies disappear and are replaced by elevated IgG antibodies, if the pathogen remains in tissues. Pathogens that have infiltrated tissue cells are no longer present in saliva and will not be wasted out in stool, thus salivary and stool cultures are not able to identify latent/dormant pathogens.

By assessing IgG antibodies against pathogens, the healthcare practitioner can see into the microscopic world of pathogens that contribute to autoimmune processes. For it is typically the silent pathogen that slowly damages the host tissues causing such autoimmune disorders as:

- Multiple sclerosis
- Guillain-Barré syndrome
- Thrombosis
- Endocarditis
- Coronary heart disease
- Myocarditis
- Type-1 diabetes
- Rheumatoid arthritis
- Irritable bowel disease
- Colitis

and additional clinical conditions such as:

- Migraine headaches
- Seizures
- Megacolon
- Gastric ulcers
- Liver abscesses

As your Premier Autoimmune Laboratory, Cyrex assesses the IgG antibodies against specific pathogenic antigens in order to help practitioners identify another category of environmental triggers that contribute to autoimmunity. By combining Array 12 with additional arrays of the Cyrex System™, a more complete picture of the autoimmune pathogenesis can elucidated.

MECHANISMS OF PATHOGEN- INDUCED AUTOIMMUNITY

A number of clinical reports and experimental studies have shown that autoimmune reactions and/or autoimmune diseases are induced in humans by environmental triggers, as summarized and reviewed by Christen and von Herrath[9] and by Delogu, *et al*.[10] Pathogens are prime candidates for enhancing autoimmune disease in susceptible individuals, because infections frequently induce strong inflammatory responses in various organs. In many cases, it is not a single infection but rather the "burden of infections" from childhood that is responsible for the induction of autoimmunity.[11] There are several major pathways, some of which are described below, through which pathogens can initiate or exacerbate autoimmunity. However, it is important to note that some pathogens, in some individuals, can also act to ameliorate autoimmunity. Thus, there appears to be a fine balance between positive versus negative effects caused by microorganisms.[9] Mechanisms involved in the initiation of a disease process might differ from mechanisms responsible for exacerbation of the established illness. Therefore, one or more of

these mechanisms, either individually or jointly, can have strong effects on the development of autoimmune reactions, which may then be followed by autoimmune diseases (**Figure 4**).

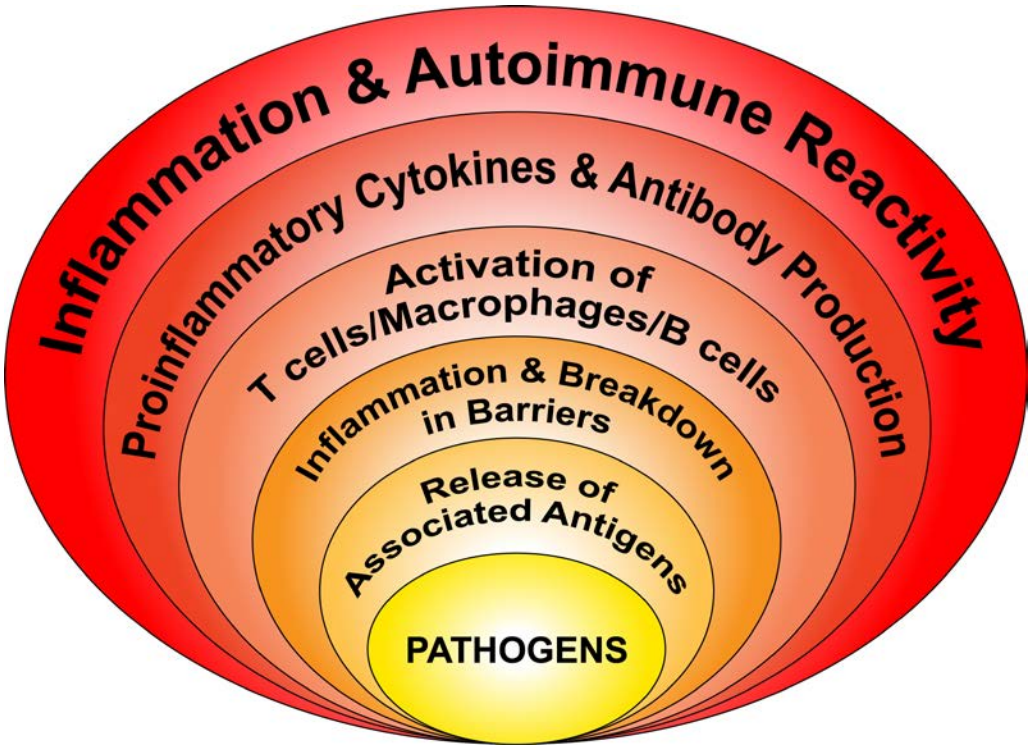

Figure 4. Induction of inflammation and autoimmune reactivities. *A tiny microbe antigen can balloon into a significant clinical condition.*

Almost every autoimmune disease is linked to one or more infectious agent and over the last half century, molecular techniques have been utilized to explore the interaction between infections and autoimmunities.[11 12 13 14] A prime example of infection associated with autoimmune disease is type 1 diabetes. Type 1 diabetes, sometimes known as juvenile diabetes or insulin-dependent diabetes, is a chronic condition in which the pancreas produces little or no insulin, a hormone needed to allow sugar to enter cells to produce energy. Classified as an autoimmune disease, type 1 diabetes, results from the destruction of pancreatic islet β-cells in genetically predisposed individuals. Environmental agents are attributed to the risk of type 1 diabetes.[15] Antibodies against glutamic acid decarboxylase 65 (GAD-65) and tyrosine phosphatase precedes the onset of the disease by 5–10 years.[16 17] Several lines of evidence link infections with type 1 diabetes:

- Enteroviruses such as rotavirus, a common cause of childhood gastroenteritis, not only shares homology with GAD-65, but can cause the precipitation of type 1 diabetes when introduced. Higher levels of anti-rotavirus antibodies are detected in sera from patients with recent onset of type 1 diabetes.[18]

- Inoculation of the virus to genetically susceptible strains of mice resulted in insulitis and diabetes, fulfilling Koch's postulates.[12]
- Both DNA and RNA viruses are capable of initiating antiviral responses that cross-react with insulin, GAD-65, and other islet cell antigens.[12 19 20]

Altering the balance of gut microbiota by using antibiotics or probiotics may also influence the development of type 1 diabetes.[21] Therefore, just as with their viral counterparts, there is sufficient, indirect, evidence that gut and other microbial agents, for example, *Mycobacterium avium*, are potential triggers for type 1 diabetes.[22 23]

Dysregulation of Immune Homeostasis

The collaboration between the innate and adaptive arms of the immune system plays a crucial role in the promotion or inhibition of autoimmune disease. Generally, to clear infections the innate immune cells can upregulate costimulatory molecules and produce a mixture of pro and anti-inflammatory cytokines such as interleukin-1-beta (IL-1β), IL-12, transforming growth factor-beta (TGF-β), IL-23, tumor necrosis factor-alpha (TNF-α), and IL-6 that regulate the adaptive arm of the immune system. However, a dysregulated immune response to environmental triggers, such as pathogens, microbiota, or their toxins, can initiate a chronic inflammatory response through activation of T-helper-1 (Th1), Th17, and TNF-α and the production of IL-17, IL-22, interferon-gamma (IFN-γ), and IL-21, resulting in inflammation, antibody production and tissue injury.[24]

A dysregulated adaptive immune system is at the core of the pathogenesis of autoimmune and other immune-mediated diseases. Hyperactivation of innate immune response affects the adaptive immune response as well as development effector T and B cells. Together, with defects in the regulatory T cells, this results in the breakdown of immune homeostasis and the development of autoimmunity.[25] For a pictorial depiction of the above, see **Figure 5**.

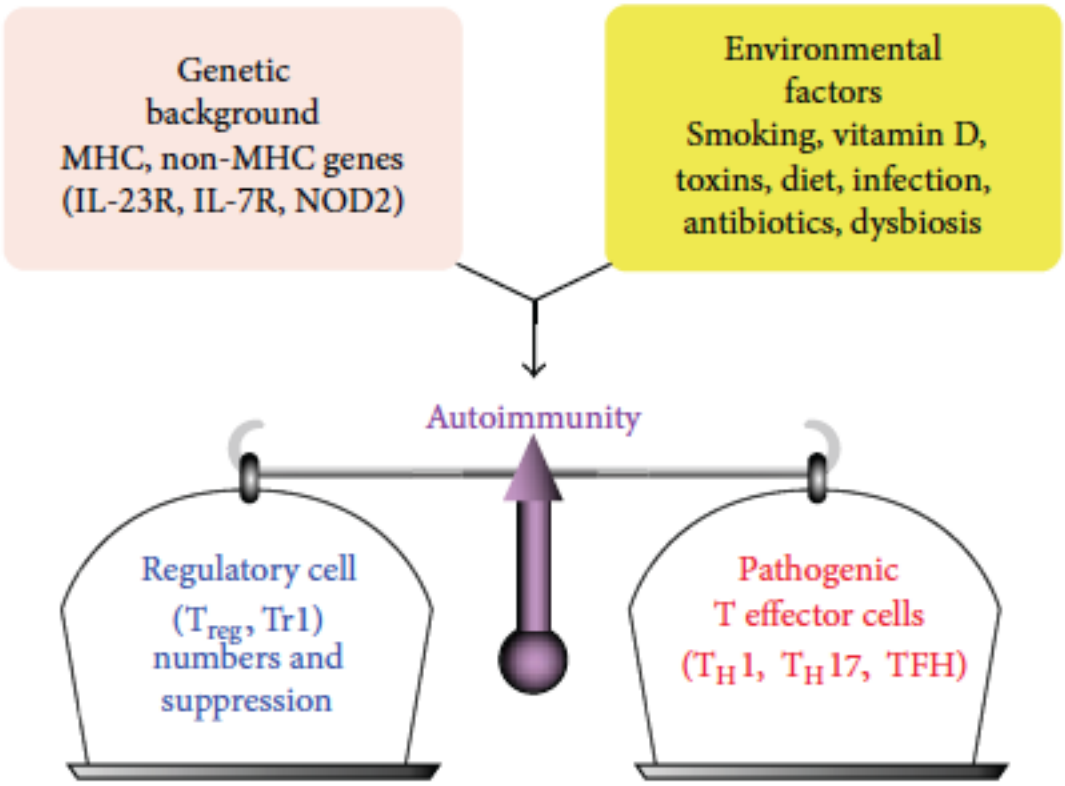

Figure 5. The balance of immunity. *A combination of host genetic factors and exposure to environmental triggers promote the development of autoimmune disease. A balance must be maintained between the regulatory T cells and the pathogenic T effector cells. From Vojdani A. A potential link between environmental triggers and autoimmunity.* ***Autoimmune Diseases****, Volume 2014, Article ID 437231, 18 pages. http://dx.doi.org/10.1155/2014/437231, 2014.*

To induce an autoimmune response in the lymph nodes, effector T cells must first acquire a defined cytokine fingerprint and then will migrate to the appropriate target organs where they initiate tissue inflammation. The effector cells that participate in the induction of autoimmunities are IFN-γ-producing Th1 cells, IL-17- and IL-22-producing Th17 cells, and IL-21-producing follicular Th (TFH) cells. It has been shown that over-activation, or expansion, of these newly discovered TFH cells causes antibody production and the development of lupus-like disease in an animal model.[26] High concentrations of circulating T cells that resemble TFH cells, have been detected in a subgroup of patients with lupus. This increased frequency of TFH cells correlated with both disease severity and end-organ damage.[27] A decrease in frequency and function of the FOXP3+TREG cell is often seen in autoimmune diseases. This decrease seems to be associated with the inflammatory environment that contributes to the dysregulation of TREG cells.[25] (see **Figure 6**)

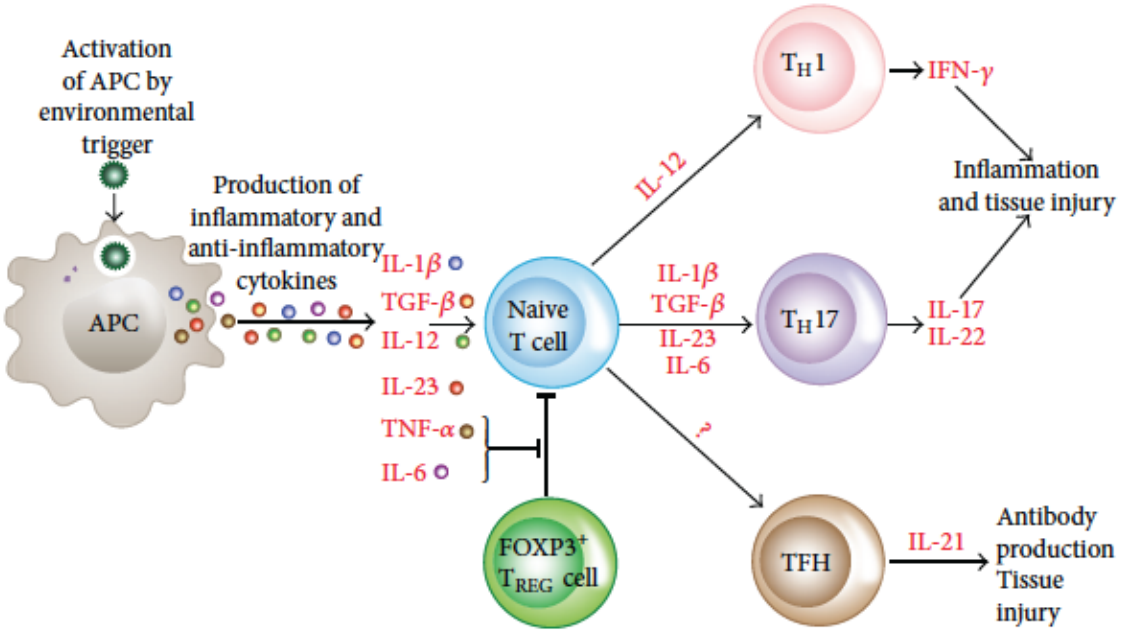

Figure 6. Differentiation of naive T cells into pathogenic effector T cells. *APCs can be activated by numerous factors, resulting in the release of cytokines that promote the differentiation of naive T cells into various subsets of pathogenic effector T cells that drive inflammation, tissue injury, and autoantibody production. Segmented filamentous bacteria (SFB) can also promote the development of Th17 cells and autoimmune responses in vivo. Proinflammatory cytokines derived from both innate and adaptive immune cells attenuate TREG cell-mediated suppression of effector T cells. From Vojdani A. A potential link between environmental triggers and autoimmunity.* ***Autoimmune Diseases****, Volume 2014, Article ID 437231, 18 pages. http://dx.doi.org/10.1155/2014/437231, 2014.*

When there is immune homeostasis, the actions of autoreactive Th1, Th17, and TFH cells are countered by FOXP3+ regulatory T cells that produce TGF-β and IL- 10. On the other hand, in an inflammatory milieu, the deletion of different transcription factors results in the generation of TREG cells that are unable to suppress the autoreactive T cells (**Figure 5**). Thus, tight control of autoreactive T cells, in particular TFH cells, by TREG cells is necessary to suppress the development of autoimmune lupus-like disease. In order to induce long-lasting remission of immune-mediated diseases, two important factors have to be in place:

1. controlling the inflammatory environment
2. boosting the frequency and function of FOXP3+ regulatory T cells.

In the Microcosm

Upon overcoming and penetrating the body's barriers, a pathogen is bombarded by neutrophils at the site of invasion. Secondary immune reactivity involves B and T cells. T cells release proteins that help stimulate the B cells. These proteins can also stimulate the death of tissue cells to prevent infection from spreading. While T cells are managing the spreading of the pathogen, B cells immediately fight the pathogen. T cells can also release chemicals which cause phagocytes, to produce responses in the body that make environments inhospitable for pathogens, such as producing a fever, which often accompanies active infections. If the pathogen evades immune attack and establishes itself in the human body, it may contribute to autoimmunity.

Molecular mimicry. A microbial antigen can include an amino acid sequence that is structurally similar to a tissue protein, providing the basic element of the mechanism referred to as molecular mimicry.[10] (See **Table 1**.) When there is similarity between the sequences of the pathogen or its agents and the body's own tissues, the host's immune system produces antibodies against the microbial-antigens and the host's self-tissues, resulting in an autoimmune reaction. Thus, T cells that are activated in response to the pathogen are also cross-reactive to self and lead to direct damage and further activation of other immune system elements. Data from the literature indicate that even a very small sequence segment or 2 or 3 amino acids with <50% similarity can induce cross-reactivity by both humoral and innate immune activation.[28 29]

Table 1. Pathogens and Tissues Cross-Reactions. Examples of bacterial and viral antigens that can cross-react with self-antigens with potentially resultant diseases.

Pathogen antigen	Cross-reactive self-antigen	Autoimmune disease
Campylobacter jejuni	Ganglioside in peripheral nerve	Guillain-Barré syndrome
Yersinia enterocolitica	Thyrotropin receptor	Thyroid autoimmunity
Borrelia burgdorferi	Leukocyte function associated antigen	Lyme arthritis
HHV-6, EBV, Rubeolla, influenza virus, and HPV	Myelin basic protein	Multiple sclerosis
Streptococcal M protein	Myosin and other heart valve proteins	Rheumatic fever

Given the vast numbers of microbial proteins and their cross-reaction with human proteins, one would expect immune responses against microbial antigens will result in autoimmunity, but this does not happen. However, an initial immune response could result in **epitope spreading** (described below), or exposure to other regions, of the same self-protein, and production of more antibodies.[30] Infection-induced autoimmunity through molecular mimicry is shown in **Figure 7**.

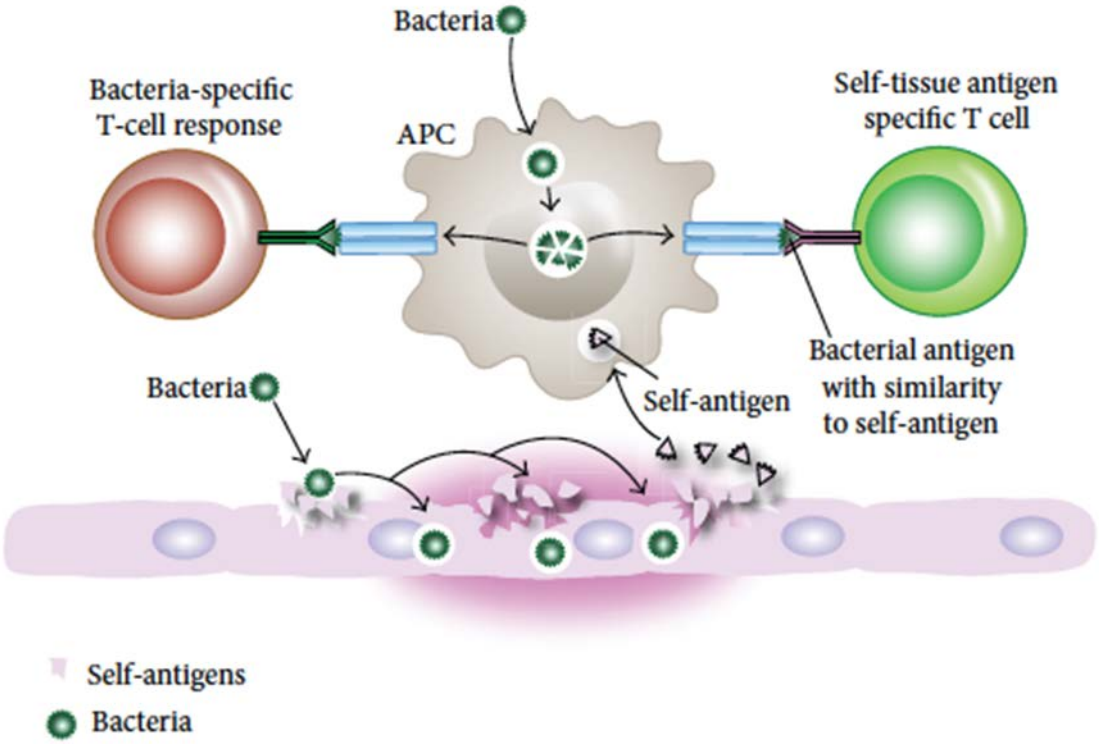

Figure 7. Mechanisms of infection-induced autoimmunity through molecular mimicry. *Bacterial induction of self-tissue antigen release and simultaneous presentation of bacterial and self-tissue antigens to T cells; activated T cells can produce antibodies against both bacterial and self-tissue antigens. From Vojdani A. A potential link between environmental triggers and autoimmunity. Autoimmune Diseases, Volume 2014, Article ID 437231, 18 pages. http://dx.doi.org/10.1155/2014/437231, 2014.*

Bystander activation*.** Bystander activation describes an indirect or non-specific activation of autoimmune cells caused by the inflammatory environment present during infection. Antigen presenting cells (APCs) that have become activated due to the inflammatory response during a pathogenic infection can stimulate the activation and proliferation of autoreactive T or B cells. In this scenario, APCs present self-antigen, obtained due to tissue destruction and/or the uptake of local dying tissue cells, to autoreactive cells.[31 32] See **Figure 8**. Furthermore, an even broader form of bystander activation occurs by cross-linking MHC class II molecules on APC with T cell receptors comprising a certain Vβ domain by ***superantigens. There are reported examples in which superantigens are involved in diseases such as experimental autoimmune encephalomyelitis (EAE, the animal model of systemic lupus erythematosus), arthritis and inflammatory bowel disease, making superantigens another mechanism by which bystander activation can initiate or exacerbate autoimmunity.[33 34 35]

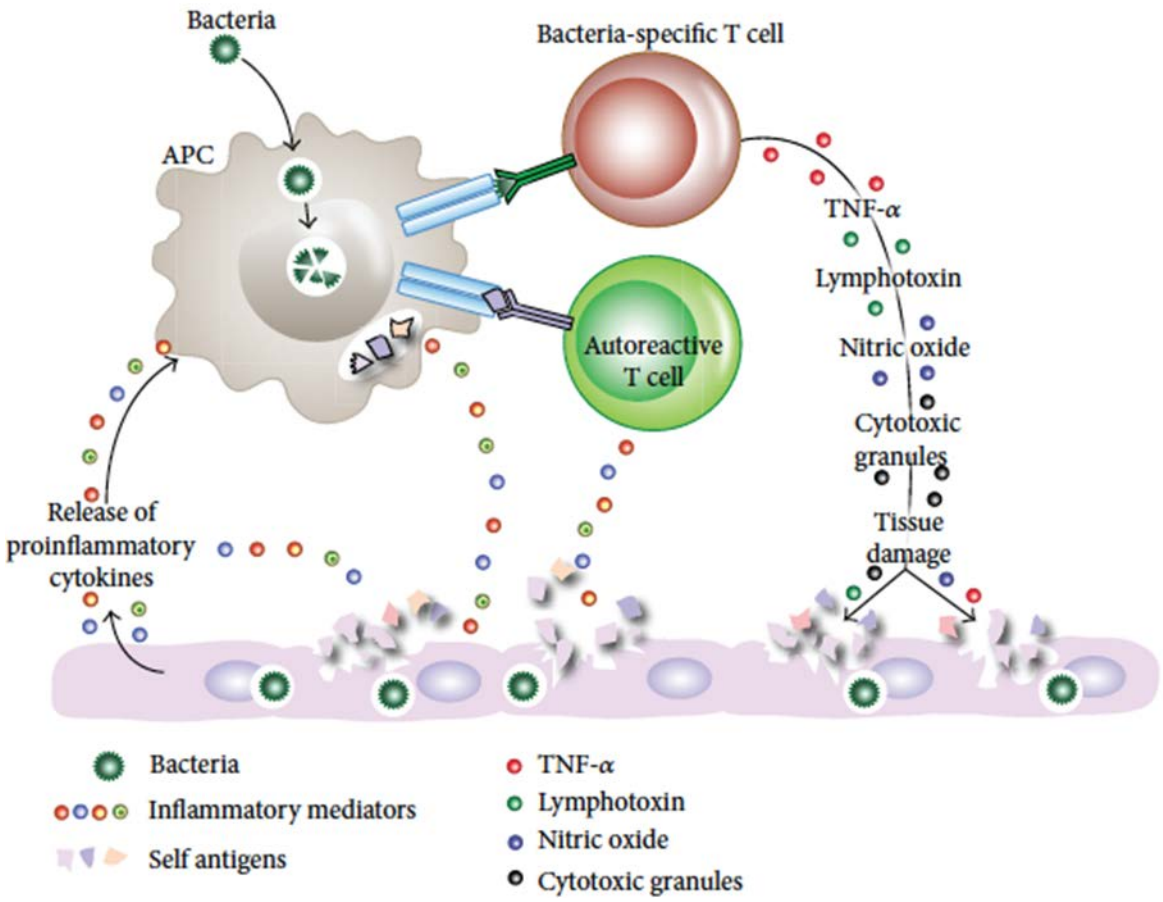

Figure 8. Microbial infection stimulates toll-like receptors (TLRs) and other pattern recognition receptors on antigen-presenting cells (APCs), leading to the production of proinflammatory mediators, which in turn can lead to tissue damage. *The release of both tissue antigens and bacterial antigens results in bacterial-specific T cells and autoreactive T cells in the process called bystander activation, which contributes to autoimmunity. From Vojdani A. A potential link between environmental triggers and autoimmunity.* ***Autoimmune Diseases****, Volume 2014, Article ID 437231, 18 pages. http://dx.doi.org/10.1155/2014/437231, 2014.*

The release of tissue proteins can activate autoreactive T cells that initially were not involved in the immune reactivity against the original infection.[11] Virally infected APCs, and the concomitantly released mediators, are able to activate autoreactive Th1 or Th17 cells in a bystander manner. Upon recognition of virally infected tissue cells, viral-specific T cells then release cytotoxic granules and cytokines such as TNF-α, IL-17, lymphotoxin, and nitric oxide. This inflammatory environment can lead to the bystander destruction of uninfected neighboring cells.

T cells that are stimulated in this manner may contain a subset recognizing specific tissue antigen.[36] Examples of superantigens are staphylococcal antigens, mycoplasma antigens, enteric-microbiota LPS, EBV, retrovirus, and many heat shock proteins. See **Figure 9**.

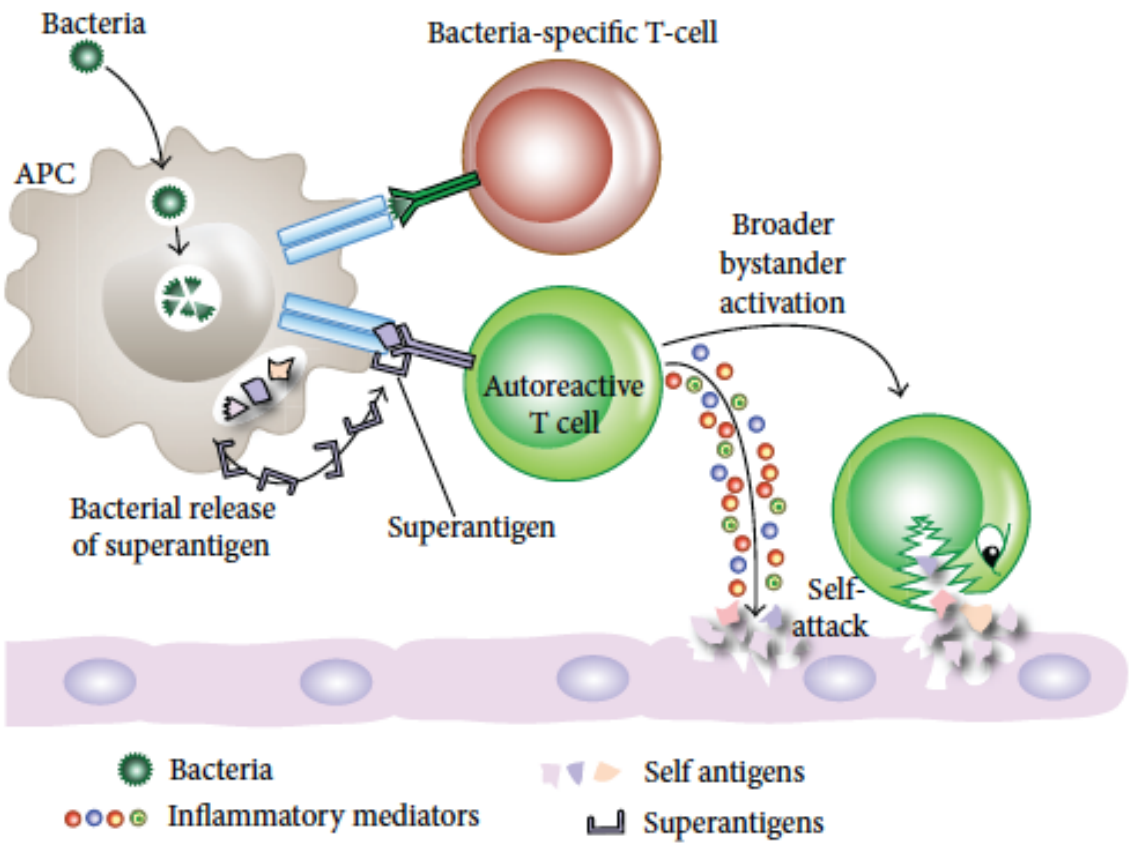

Figure 9. Superantigens and autoimmunity. *Infection can lead to the release of superantigens, which can cross-link between MHCII and TCR, causing broader bystander activation, some of which may be specific for self-antigens, leading to attack on self-tissues. From Vojdani A. A potential link between environmental triggers and autoimmunity.* ***Autoimmune Diseases****, Volume 2014, Article ID 437231, 18 pages. http://dx.doi.org/10.1155/2014/437231, 2014.*

Some superantigens do not cause autoimmune reactivity, but are involved in the exacerbation of arthritis, irritable bowel disease, and other disorders.[37]

Epitope spreading. Epitope spreading refers to the ability of the B and T cell immune response to diversify both at the level of specificity and V gene usage.[38 39 40] It is a scenario in which an immune response that is initiated by various stimuli, including microbial infection, trauma, transplanted tissue or autoimmunity, spreads to include responses directed against a different portion of the same protein (intramolecular spreading) or a different protein (intermolecular spreading).[10 41] As reviewed by Jones et al.[25] epitope spreading in EAE has been observed to involve:

- T cells specific for additional epitopes on the inducing myelin protein Ag (intramolecular spreading)
- T cells specific for different myelin proteins (intermolecular spreading)
- T cells restricted by a different H-2 allotype than that of the inducing T cells.

Activation of naive T cells through endogenous self-priming to myelin tissue proteins may occur in lymph nodes and has been proposed as the disease-dependent mechanism responsible for epitope spreading.[42]

Epitope spreading can result from a change in protein structure. One such example is protein citrullination, the changing of an amino acid from arginine to citrulline. This can result not only in immune reaction against the original protein or its citrullinated form, but also against other citrullinated proteins.

Epitope spreading is demonstrated in rheumatic fever. In this clinical scenario, a chronic autoimmune response against streptococcal M protein and heart valve tissue can result in immune response against collagen or laminin. This immune response against collagen or laminin is no longer specific to the bacterial M protein or its cross-reactive tissue protein. This mechanism of infection-induced autoimmunity through epitope spreading is shown in **Figure 10**.

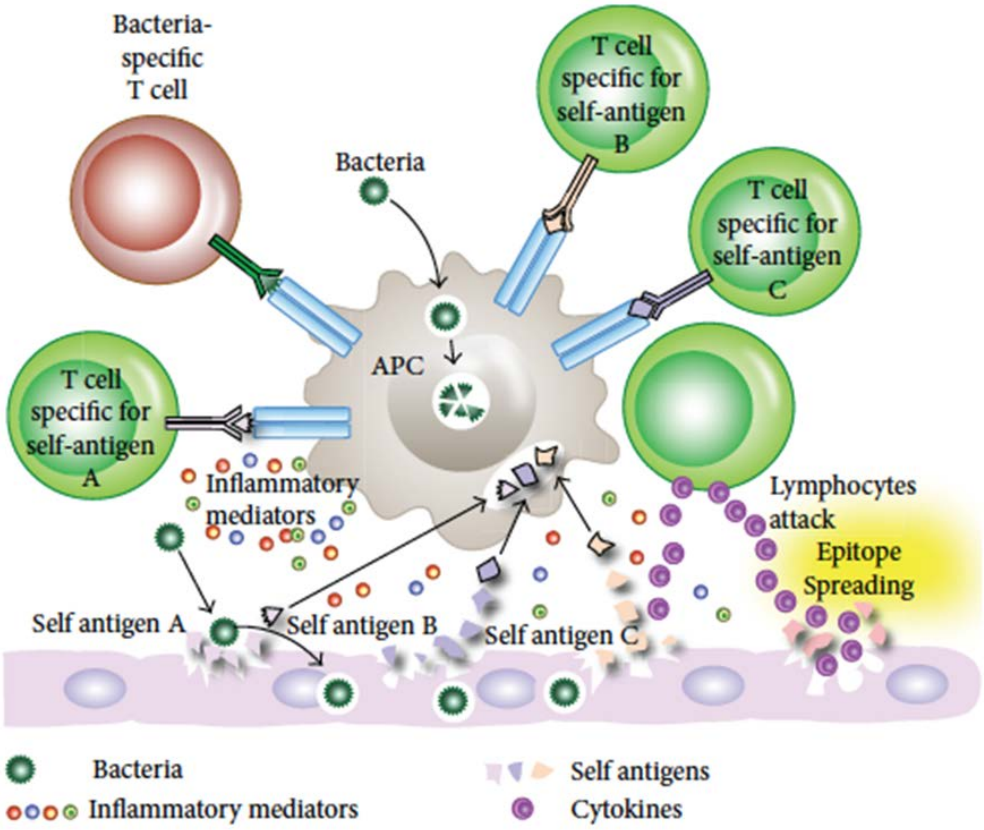

Figure 10. Bacterial infection induces release of tissue antigen and presentation of bacterial and self-tissue antigens resulting in the induction of autoreactive T cells. *T cells and inflammatory mediators cause the release of more self-antigens which differ from the original antigens. T-cell responses can then spread to involve T cells specific to other self-antigens. This T-cell response against different epitopes results in antibody production against multiple tissue antigens. From Vojdani A. A potential link between environmental triggers and autoimmunity.* ***Autoimmune Diseases****, Volume 2014, Article ID 437231, 18 pages.http://dx.doi.org/10.1155/2014/437231, 2014.*

Persistent Infection and Polyclonal Activation of B Cells. In many autoimmune diseases, such as lupus, RA, type 1 diabetes, and MS, B-cell functions are closely correlated with disease activity. Antibodies produced by B-cell-derived plasma cells contribute significantly to disease pathogenesis.[30] In these and other disorders, prolonged infectivity with a virus, such as Epstein-Barr virus, viral proteins, or viral genomes can lead to autoimmunity by the constant activation and proliferation of B cells. After a long period of polyclonal B-cell activation, sometimes monospecific clones can emerge, accompanied by very high levels of antibody production and the formation of circulating immune complexes. Finally, this mixture of polyclonal antibodies and immune complexes may cause the autoimmune disease,[43] as shown in **Figure 11**.

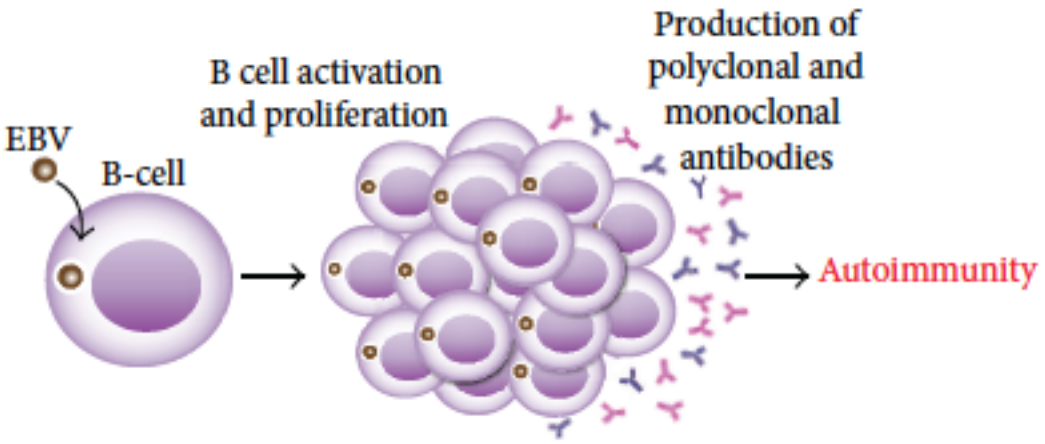

Figure 11. Infections, B cells, and autoimmunity. *Prolonged infection with a virus, such as EBV, can lead to constant activation and proliferation of B cells, resulting in the production of monoclonal and polyclonal antibodies as well as immune complexes, causing autoimmune disease. From Vojdani A. A potential link between environmental triggers and autoimmunity.* ***Autoimmune Diseases****, Volume 2014, Article ID 437231, 18 pages. http://dx.doi.org/10.1155/2014/437231, 2014.*

Research is underway to elucidate additional ways in which pathogens trigger or exacerbate autoimmune reactivities. Keep in mind that some microorganisms are helpful to the human body and can even ameliorate autoimmune reactivity in some individuals. To learn more about the protective mechanisms of pathogens, please read Christen and von Herrath's excellent review article, "Infections and Autoimmunity – Good or Bad?" in The Journal of Immunology.[9]

In the Microcosm

The most common mechanisms by which pathogens trigger autoimmune reactivity are:

- Molecular mimicry
- Induction of inflammatory cascades

Clinical Aspects of Array 12

Environmental triggers of autoimmunity include stress, dietary proteins and peptides, chemicals and pathogens. Array 12 – Pathogen-Associated Immune Reactivity Screen™ assesses the fourth category of environmental triggers. By combining Array 12 with other pertinent arrays, the picture of an individual's autoimmune pathology can become clear. The detection of elevated IgG against pathogens indicates the patient harbors pathogens beyond the acute infection stage. Detected pathogens may be in a latent or chronic reactivating state and therefore contributing to autoimmune reactivity. Array 12 does not identify pathogens in acute infection stage. If you need to assess acute infections please utilize an IgM evaluation of pathogens.

In the Microcosm

Array 12 assesses latent or chronic reactivating pathogens. It is not helpful in identifying acute infections

ARRAY 12 ANTIGEN ROLL CALL

The Array 12 – Pathogen-Associated Immune Reactivity Screen™ (PAIRS) consists of bacteria, viruses, stealth organisms, parasites and fungi. See **Figure 12**.

Figure 12. Array 12 – Pathogen-Associated Immune Reactivity Screen™ (PAIRS) antigens. *This one of a kind array identifies a variety of pathogens that have remained in the body after the initial, acute infection stage.* ☐ *- Oral pathogens;* ☐ *- Gastrointestinal pathogens;* ☐ *- Gastrointestinal parasites;* ☐ *- Bacterial and stealth pathogens;* ☐ *- Environmental molds;* ☐ *- Viral pathogens;* ☐ *- Tick-borne pathogens.*

Individual white papers for these antigens are available on the professional side of the Cyrex website. Each white paper provides specific and detailed information about the antigen, its mechanisms of colonization within the human host and known mechanisms of autoimmunity. Below are definitions of each antigen; please review the white papers for more in depth education on Array 12 antigens.

Porphyromonas gingivalis. Oral bacterium *P. gingivalis* has been well-documented as a mediator of periodontal disease. Furthermore, hosts harboring this pathogen have been shown to have greater risk for rheumatoid arthritis. Researchers are elucidating the mechanisms by which *P. gingivalis* contributes to the pathogenesis of arthritic and their related disorders. Upregulation of intestinal lipopolysaccharides and subsequent inflammation, as well as citrullination of alpha-enolase, which shares homology with human tissue α-enolase, are described mechanisms of autoimmunity. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

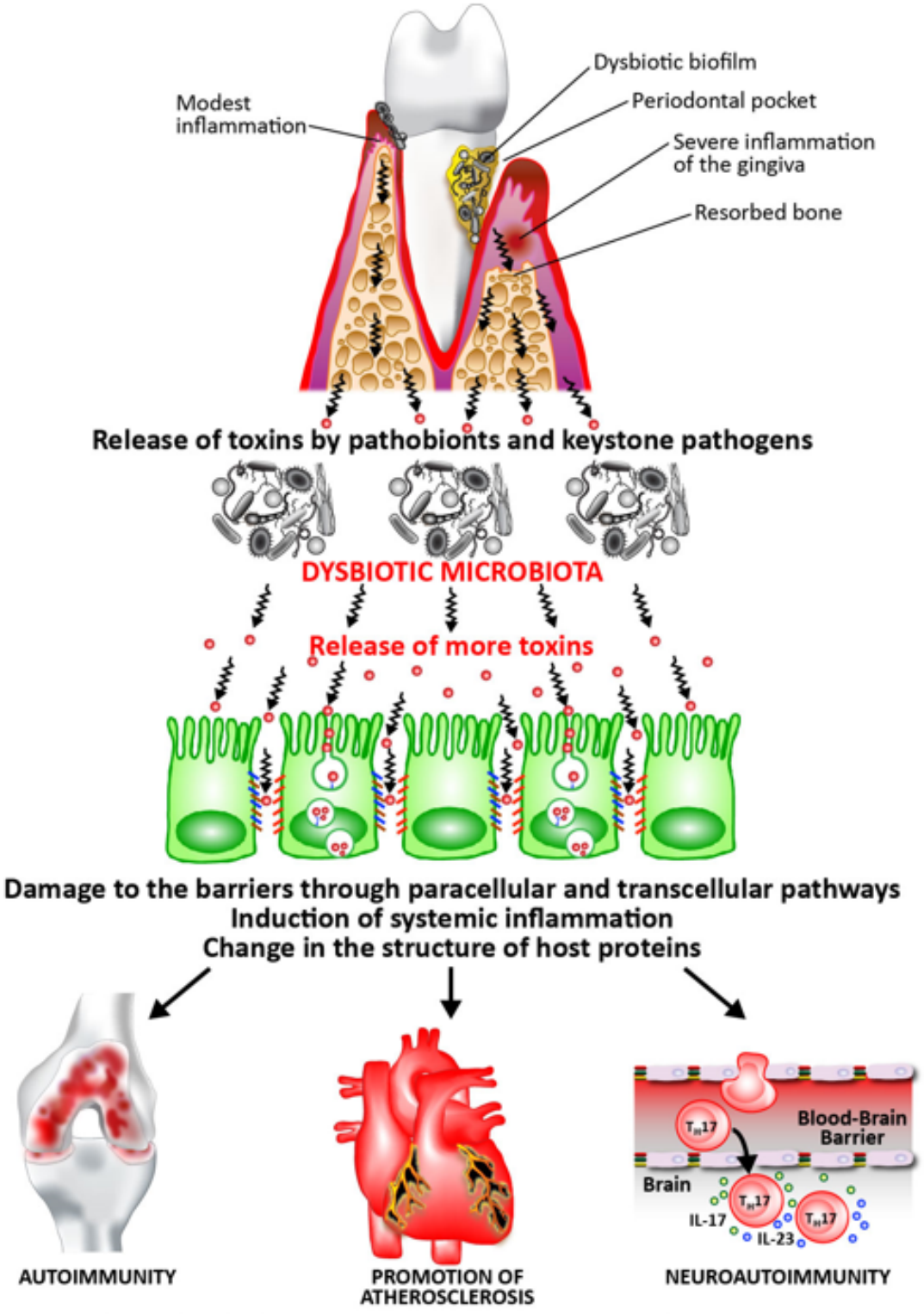

Figure 13. Mechanism of Oral Pathogens in Autoimmunity. *Keystone and pathobionts such as P. gingivalis and S. mutans can trigger periodontitis, which alters microbiota, which leads to dysbiosis, opening of intestinal tight junctions, systemic inflammation and autoimmunity.*

Streptococcus mutans. *S. mutans* is a gram positive bacterium commonly found in the human oral cavity. It is known to promote dental caries. *S. mutans* has been shown to elicit inflammation by stimulating cytokine production in the dental pulp below caries. When this inflammation reaches the intestines, it can contribute to dysbiosis, breakdown of intestinal barrier structures and the infiltration of *S. mutans* immunogens into circulation. Antibodies against *S. mutans* have long been recognized as cross-reactive to human heart tissues and thus, suspected of playing a role in heart disorders. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Helicobacter pylori. *H. pylori*, a gram-negative bacterium colonizes the gastrointestinal system, where is interferes with intestinal barrier functions, induces inflammatory responses and can contribute to autoimmunity. Mechanisms of autoimmunity include molecular mimicry, polyclonal activation, epitope spreading, bystander activation and super antigen release. *H. pylori* has been implicated in disorders of the thyroid, liver, joints and nervous system. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

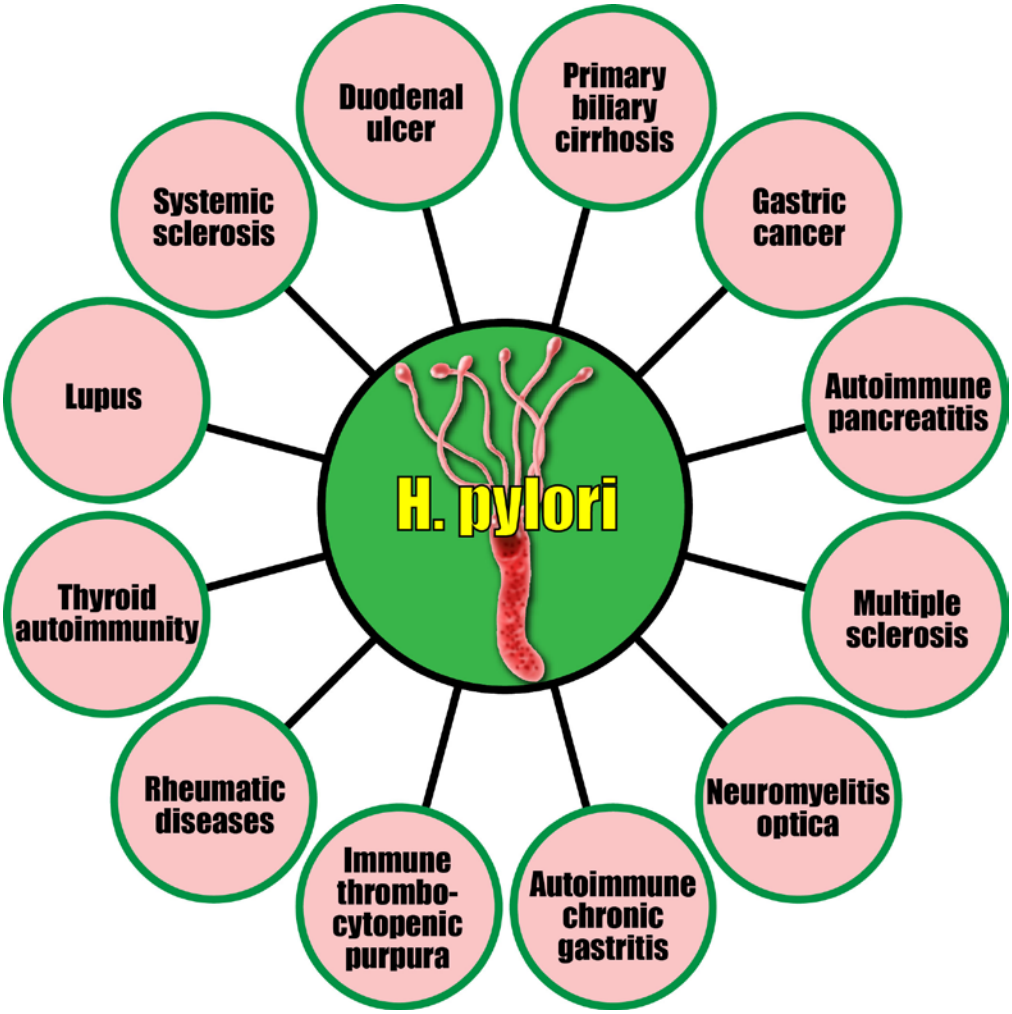

Figure 14. Disorders Associated with Helicobacter pylori. *Depending on the patient's susceptibility, H. pylori trigger a variety of disorders from gut to brain.*

Campylobacter jejuni. *C. jejuni* is a gram-negative bacterium that causes severe gastroenteritis. Due to *C. jejuni*'s ability to produce lipoligosaccharides, the bacteria are able to invade intestinal epithelial cells. Beyond the gut wall, *C. jejuni* has been implicated in disorders such as arthritis and Guillain-Barré syndrome. The severity of these disorders makes *C. jejuni* an important environmental trigger to assess while working up certain autoimmune patients. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

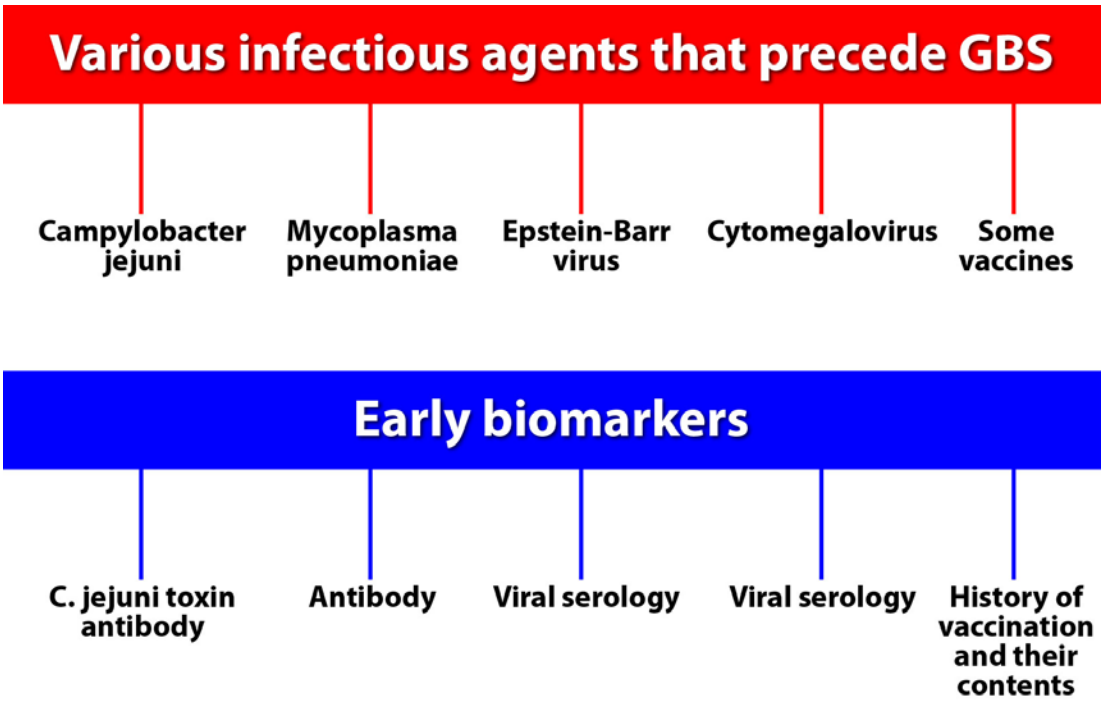

Figure 15. The Relationship Between Infectious Agents and Biomarkers of GBS. *There is an association between various infectious agents that precede Guillain-Barré syndrome and biomarkers that can be used to prevent neurological symptomatologies. When biomarkers are detected at the first stages of autoimmunity, protocols can be implemented that can arrest the autoimmune process and prevent the onset of autoimmune disease.*

Yersinia enterocolitica. *Y. enterocolitica* is a gram-negative, bacillus-shaped bacterium. *Y. entrocolitica* can be short-lived as an infection. However, even if the infection is treated successfully, due to the action of various bacterial toxins and mimicry with human tissue, continued immune responses against these toxins may result in various inflammatory and autoimmune disorders, such as inflammatory bowel disease, autoimmune thyroid disease, uveitis, Lyme-associated disorders and even reactive arthritis. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

YOP:	D	A	L	G	N	V	T	S
	|	|		|		|		|
TSH-R:	D	A	F	G	G	V	Y	S

Figure 16. Sequence Homology Between YOP and TSH-R. *There is a similarity between potential epitope of Yersinia enterocolitica outer membrane protein (YOP) and thyroid stimulating hormone receptor (TSH-R) which may account for a cross-reactivity mechanism of autoimmunity between Y. enterocolitica and thyroid tissues.*

Clostridium difficile. *C. difficile* is the leading cause of antibiotic-associated nosocomial diarrhea and colitis in the industrialized world. This gram-positive bacterium can reside in the human host without triggering serious clinical conditions, however, when the colonized bacteria produce toxin A and toxin B, the resulting changes in gastrointestinal pH and cytoskeletal structures of the barrier, serious disorders can occur. Pathogenic *C. difficile* has been linked to colitis, irritable bowel disease and liver disorders. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

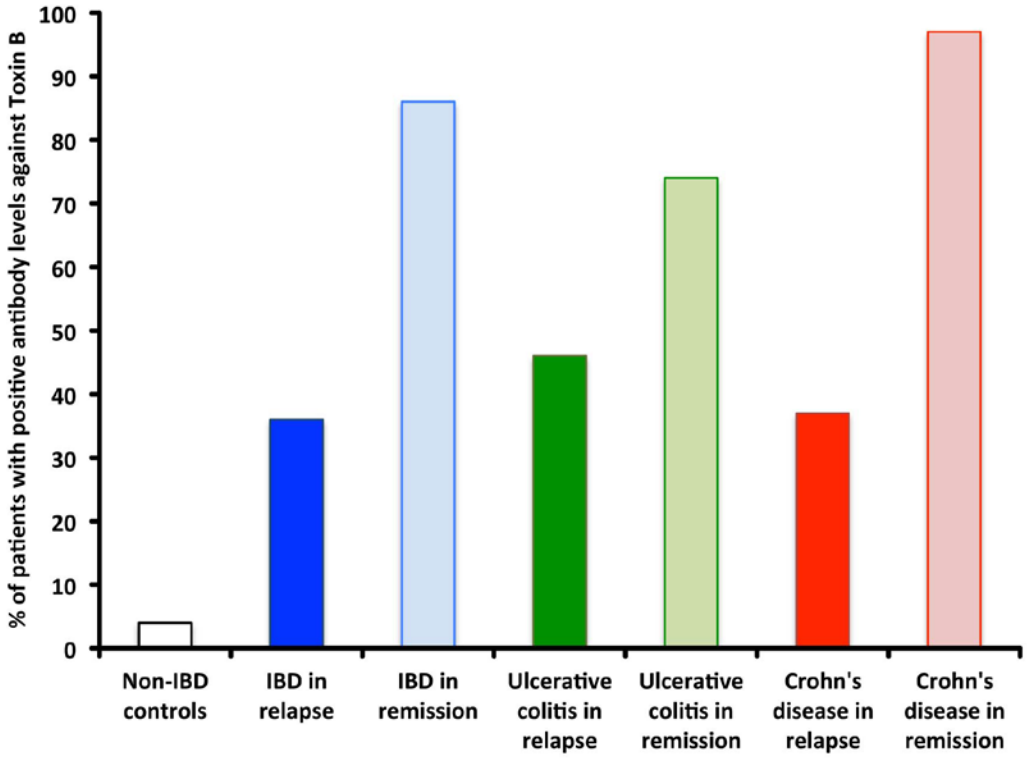

Figure 17. The Proportion of Patients with IBD, Crohn's and Ulcerative Colitis with IgG anti-*C. difficile* Toxin B Compared to Non-IBD Controls. *Modified from Shakir FA et al., Determination of serum antibodies to Clostridium difficile toxin B in patients with inflammatory bowel disease. Gastroenterol Hepatol (N Y); 8(5): 313–317, 2012.*

Candida albicans. *C. albicans* is a human commensal yeast. By penetrating the intestinal barrier this pathogen is able to thrive in the human host. Its inflammatory effect in the gastrointestinal tract opens the intestinal barrier, putting tissue and organs at risk for autoimmunity. *Candida* has been shown to cross-react with a variety of human tissues and thus, when *Candida* or its antigens reach the blood stream, the antibodies produced against it may turn on self-tissue proteins. The end result can be autoimmunity. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

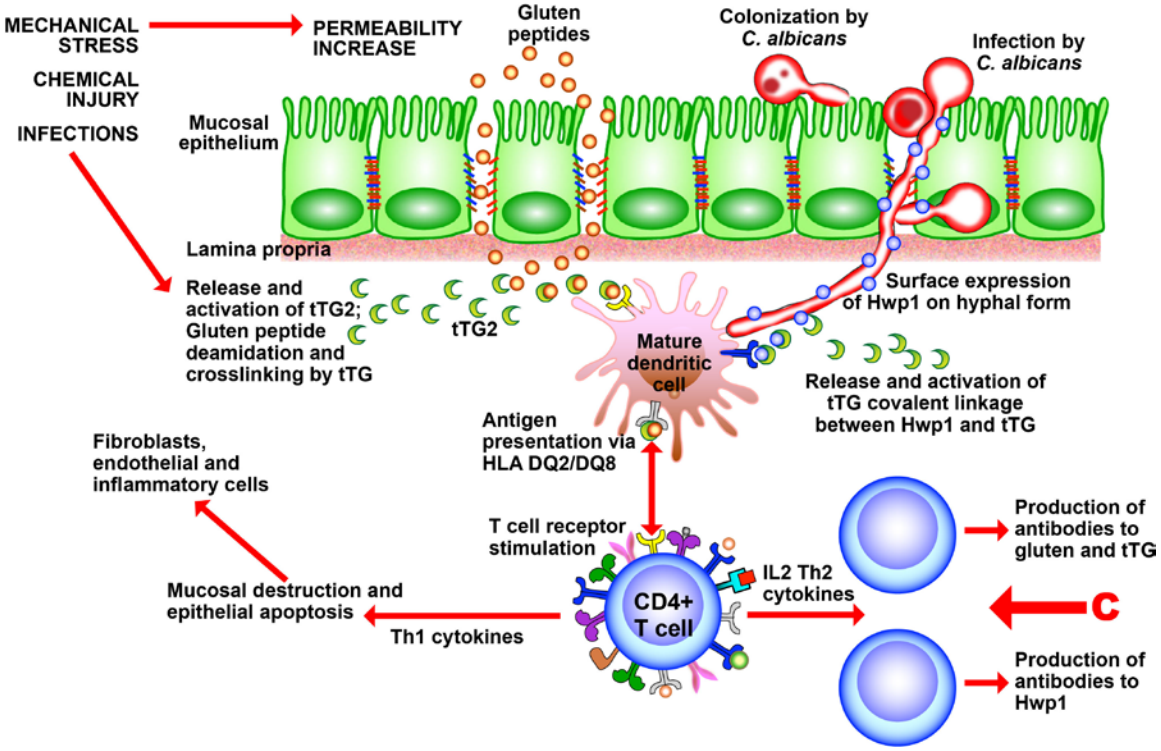

Figure 18. Mechanisms by which *C. albicans* Induces Celiac Disease. *As an environmental trigger, C. albicans can inflame intestinal tight junctions, thus breaking the structures and releasing tissue proteins such as transglutaminase-2 into the bloodstream. Broken tight junctions allow gluten family peptides to cross into the lamina propria. C. albicans or its antigens can then ignite a systemic immune reaction resulting in cytokine and antibody production against gluten peptides, tTG2 and C. albicans antigens.*

Rotavirus. Rotavirus is a double-stranded RNA virus that is commonly associated with gastroenteritis in children. Repeated infections with rotavirus can lead to viral replication in intestinal cells. Changes in intestinal cells leads to intestinal barrier dysfunction, increased intestinal permeability and the easy translocation of environmental immunogens into circulation. Pathogenic rotavirus has been linked to various disorders such as Celiac disease and type 1 diabetes. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

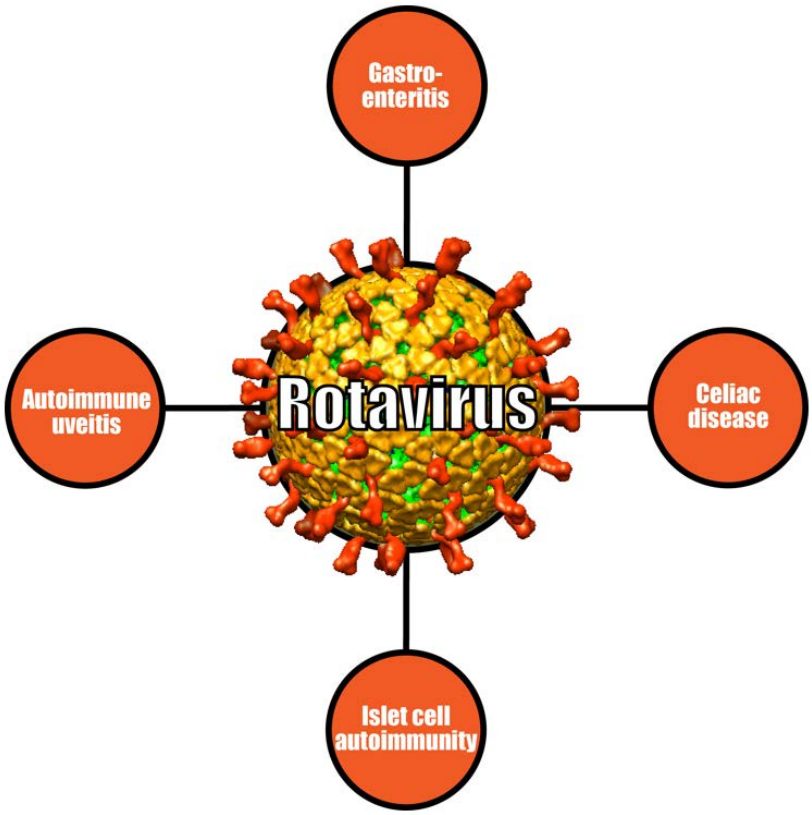

Figure 19. Disorders Associated with Rotavirus. *Rotavirus has been shown to play a role in gastrointestinal disorders, but can also reach the pancreas and even the eye and trigger autoimmunity against tissues associated with those organs.*

Entamoeba histolytica. *E. histolytica* invasion may contribute to T-helper-2 bias and antibody production particularly against *E. histolytica* lectins and their association with tissue antigens such as phospholipids, actin and ANCA. By penetrating the intestinal tissues, *E. histolytica* is able to disturb tight junction assemblies, thereby opening the intestinal tight junctions and putting the body at risk for autoimmunity. Once in the bloodstream, *E. histolytica* may trigger autoimmunity against neurological or bone tissues, due to its homology with gangliosides and skeletal actin. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Figure 20. Homology Between E. histolytica and Actin. *There is significant homology between E. histolytica and human tissue antigens. Modified from Edman E et al., A virulent strain of E. histolytica. Proc Natl Acad Sci; 84:3024-3028, 1987.*

Giardia lamblia. *G. lamblia* is a flagellated protozoan parasite that colonizes and reproduces in the small intestine. *G. lamblia* causes giardiasis. Giardiasis does not spread via the bloodstream, nor does it spread to other parts of the gastrointestinal tract. Giardiasis remains in the lumen of the small intestine. Chronic infection with *Giardia* may abate and the patient could become asymptomatic. Asymptomatic individuals may become reservoirs for spreading the infection. Antibodies against *G. lamblia* may cross-react with human tissue antigens such as tubulin, actin, actinin, tropomyosin and others. The end result may be autoimmunity against those tissues. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

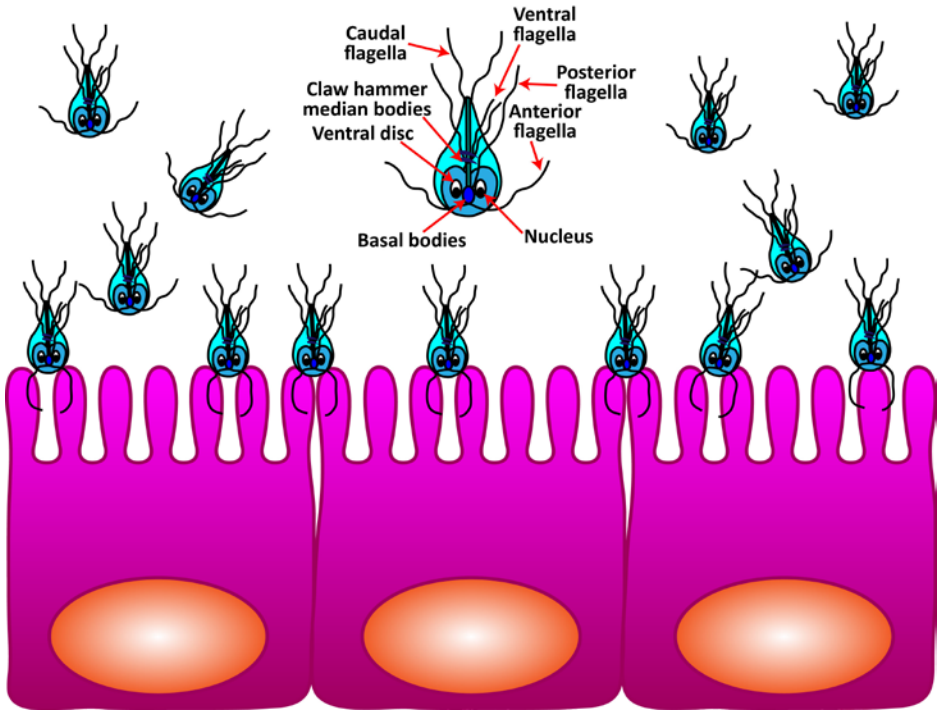

Figure 21. *Giardia lamblia* Trophozoites Emerge From the Cysts in the Duodenum and Attach to the Small Intestinal Mucosa. *Modified from Faubert G, Immune response to Giardia duodenalis. Clin Microbiol Rev; 13:35-54, 2000.*

Cryptosporidium parvum. *C. parvum* is a protozoan parasite that can cause gastrointestinal illness with diarrhea in humans. Through various mechanisms the parasite can manipulate the host cytoskeleton proteins, including rearranging tropomyosin-5 protein, actinin, villin, ezrin, at the site of infection. This restructuring of proteins allows the parasite to infiltrate intestinal cells. Due to antigenic similarity between *C. parvum*, actin and tropomyosin structures, these antibodies may cross-react with human tissues resulting in autoimmunity associated with parasitic infections. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Blastocystis hominis. *Blastocystis hominis* (*B. hominis*) is a unicellular protozoan found in the large intestine of humans. *B. hominis* is the most prevalent single-celled eukaryotic organism found in humans. It is a causative pathogen in irritable bowel disorders and the toxins released by *B. hominis* can contribute to fibromyalgia. This presence of *B. hominis*-specific immunoglobulins in the serum samples suggests

that the immune action against this parasite is not limited to the intestinal level. Antibodies to the pathogen can be found in both symptomatic and asymptomatic individuals, therefore, it is still unclear whether *B. hominis* is a truly pathogenic organism, or a commensal, or perhaps, is capable of being a pathogen in specific circumstances. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Emerging Infectious Diseases, 2014; 20(4):581-589.

Cryptosporidium in Swedish, public water supply – study revealed:

- Gluten intolerance was identified as one of the risk factors for acquiring cryptosporidiosis.
- IBD, lactose intolerance, or gluten intolerance and young age were significantly associated with more days of diarrhea.
- Gluten intolerance remained a risk factor after results were controlled for age, sex and residence.
- The mechanism by which gluten intolerance might constitute a risk factor for cryptosporidiosis is unknown.

Human HSP-60 + Chlamydia HSP-60. Heat shock protein 60 (HSP60) is a mitochondrial chaperonin that plays a role in the transportation and refolding of proteins from the cytoplasm into the mitochondrial matrix. HSP60's amino acid sequence bears a similarity to its homolog in plants, bacteria, and humans. Chlamydia is an obligate intracellular bacterium. Chlamydia HSP60 (C.Hsp60) is associated with the outer membrane complexes of Chlamydia and appears to be responsible for proinflammatory pathologic manifestations. Autoimmune disease can be induced by specific antigens such as HSPs, since antibodies against bacterial HSPs can cross-react with human HSP60 and induce cytotoxic damage to the stressed endothelial cells. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Figure 22. Region of Similarity Between HSP60, Thyroglobulin and Thyroid Peroxidase Amino-Acid Sequences. *Modified from Marino Gamazza A et al., Elevated blood Hsp60, its structural similarities and cross-reactivity with thyroid molecules, and its presence on the plasma membrane of oncocytes point to the chaperonin as an immunopathogenic factor in Hashimoto's thyroiditis. Cell Stress Chaperones; 19(3):343-53, 2014.*

Chlamydias. Chlamydias are obligate intracellular pathogens. *Chlamydia pneumoniae* (*C. pneumoniae*) is a human pathogen that infects the respiratory tract and is responsible for some cases of community-acquired pneumonia. *Chlamydia trachomatis* (*C. trachomatis*) is a human pathogen known to cause some genital tract and ocular infections. Chlamydias can have far-reaching manifestations. Long-term harboring of Chlamydias has been implicated in neuroautoimmunities such as multiple sclerosis and autism spectrum disorders. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

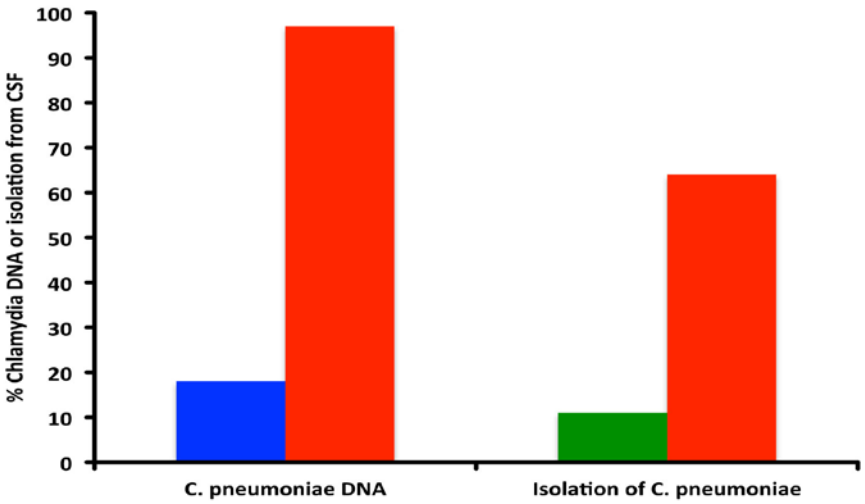

Figure 23. Percentage of detected DNA of *C. pneumoniae* in Patients with Neurological Disorders ■ Versus MS Patients ■, and Isolation of *C. pneumoniae* from CFS or Controls ■ and Patients with MS ■. *Modified from Sriram S et al., Chalmydia pneumoniae infection of the central nervous system in multiple sclerosis. Ann Neurol; 46:6, 1999.*

Streptozymes. Streptozymes (NADase, DNase, streptokinase, streptolysin O, and hyaluronidase) are extracellular products, or exoantigens, of the streptococcus bacteria. Streptococcal antigens can bind to specific receptors on human cells and if found to pharyngeal or dermal epithelial cells, tissue penetration by the pathogen can occur. In this scenario, a throat culture will not detect the pathogen, while a serological antibody assay will. Streptozyme antibodies are markedly increased in PANDAS and OCD disorders, as well as rheumatic heart disorders and reactive arthritis. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Tissue antigens that react with monoclonal antibodies made against Streptococcal antigens	
• Actin	• Laminin
• Vimentin	• Collagen
• Keratin	• *N*-acetyl-β-D glucosamine
• Myosin	• Double-stranded DNA
• Tropomyosin	• Heat-aggregated IgG or RF
• Glomerulum	• Endothelial cell

Streptococcal M protein. M Protein is an extracellular product of *Streptococcus* that contributes to the pathogenicity of the gram-positive bacterium. Antibody response against streptococcal M protein and its reaction with myosin may result in endothelial cell damage and the release of inner valve proteins. This results in antibody production against collagen, vimentin, elastin and laminin, which may contribute to the pathogenesis of streptococcal-associated disorders. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Mycoplasmas. *Mycoplasma* refers to a genus of bacteria that lack a cell wall, which makes them immune to common antibiotics such as penicillin. *Mycoplasma pneumoniae, Mycoplasma arthritidis* and *ureaplasma* are common human pathogens. The lack of rigid cell wall allows mycoplasmas to have direct and intimate contact with the cytoplasmic membrane of the host cell leading to cell fusion and delivery of lipid-associated membrane proteins (LAMPS). LAMPS are highly antigenic and hence are major mechanisms of *Mycoplasma*-induced autoimmune reactivity. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

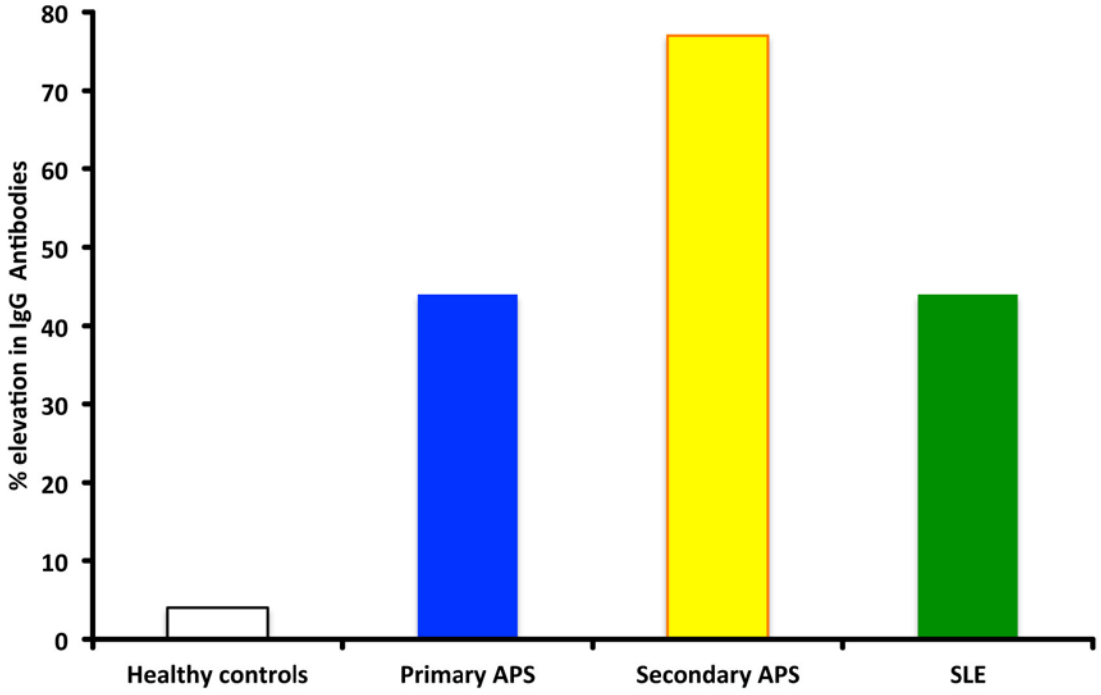

Figure 24. Measurement of IgG antibodies in patients with SLE and in healthy controls. *From data in Cahill JF et al., Role of biological mimicry in the pathogenesis of rat arthritis induced by Mycoplasma arthritidis. Infec Immun; 3:24-35, 1971.*

Acinetobacter. *Acinetobacter* is a non-motile, gram-negative bacterium. *Acinetobacter* may cause infections of the lung, urinary tract, bloodstream or surgical wounds. Due to cross-reactivity with major

neurological tissues, *Acinetobacter* has been shown to play a role in multiple sclerosis. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

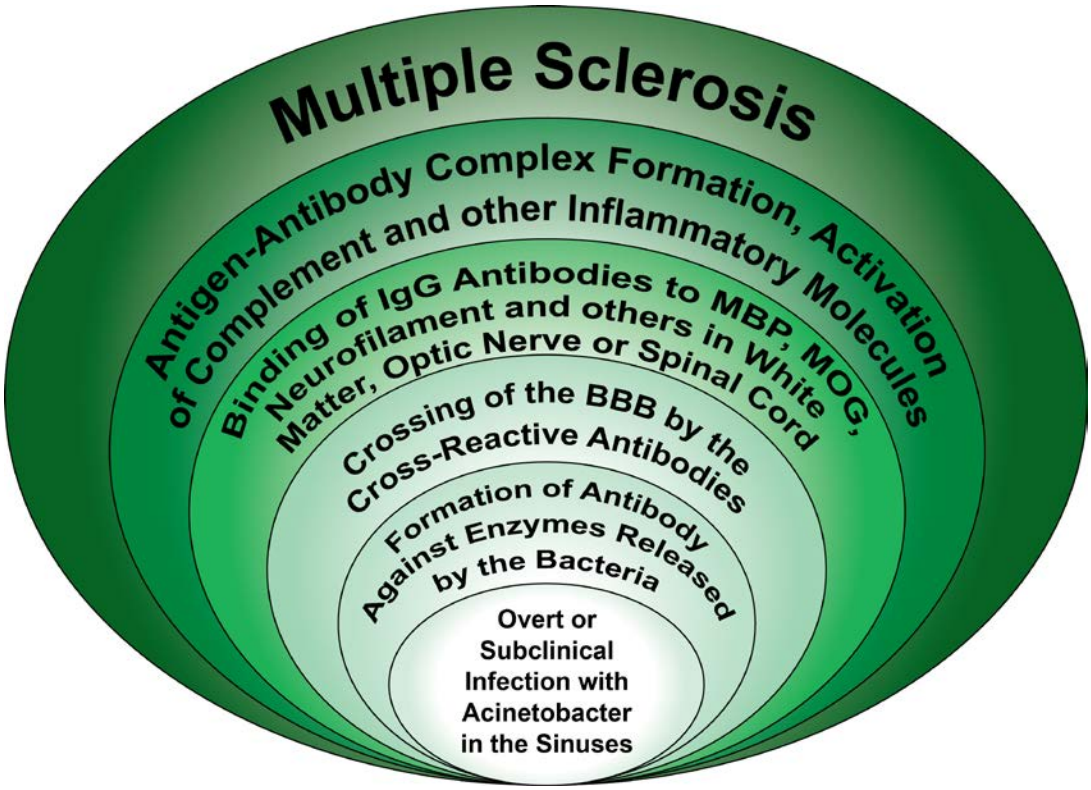

Figure 25. Measurement of *Acinetobacter* Antibodies Could be used as a Marker of Disease Activity. *Modified from Ebringer A et al., Acinetobacter immune responses in multiple sclerosis: etiopathogenetic role and its possible use as a diagnostic marker. Arch Neurol; 62(1):33-36, 2005.*

Klebsiella. *Klebsiella* are gram-negative, facultative anaerobic, non-motile, rod-shaped bacteria. Array 12 assesses immune reactivity to *Klebsiella pneumoniae, Klebsiella oxytoca* and *Klebsiella pneumoniae uti*. *Klebsiella* is one of the common hospital-acquired pathogens. The cross-reactivity between *Klebsiella* and collagen in the uvea, may explain its involvement in uvetits and iritis. The mechanism of molecular mimicry could be applied to ankylosing spondylitis, rheumatoid arthritis and Crohn's disease, after infection with causative microbes. After gut mucosal activation by *Klebsiella* antigens and production of secretory anti-*Klebsiella* antibodies, repeated *Klebsiella* infections in the large intestine of susceptible individuals could lead to increased levels of *Klebsiella* IgG antibodies in the blood. When these IgG anti-*Klebsiella* antibodies reach a certain limit, they will activate the complement cascade and complement components, plus other factors causing tissue injury leading to autoimmune reactivity. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

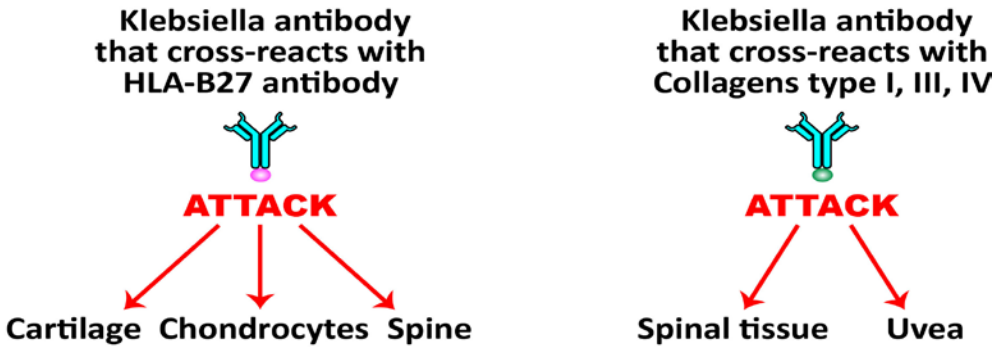

Figure 26. The types of cross-reactive antibodies produced after infection with *Klebsiella* determines the location of the lesion. *When Klebsiella antibody cross-reacts with HLA-B27 antibody, the end result could be an autoimmune attack against joint tissues. When Klebsiella antibody cross-reacts with collagens, the end result could be spinal or eye autoimmunity.*

Mycobacterium avium. *Mycobacterium avium* (*M. avium*) is a gram-positive, slow-growing bacteria with high guanine and cytosine content. It is present mainly in cattle and transmitted to humans by drinking unpasteurized animal milk. *M. avium*, *M. bovis* and *M. tuberculosis* are the most common human acquired mycobacteria. Drinking water is the most common source of *M. avium* by humans. By drinking two liters of water per day, after a little over two months, an individual could have ingested pathogenic levels of microorganisms. When saturation levels are reached there is an increase in gastrointestinal autoimmune disorders such as ulcerative colitis and Crohn's disease. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

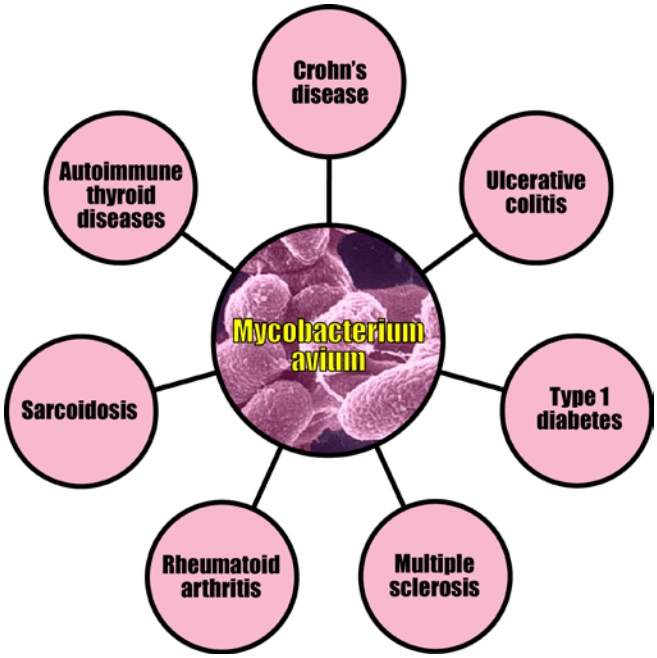

Figure 27. Mycobacterium avium Associated Disorders. *Depending on the patient's genetic susceptibility, M. avium infection may trigger autoimmunity in a variety of tissues.*

Aspergillus. *Aspergillus* is the genus of asexual spore-forming mold species common in many climates. It is found in soil, water and air. *Aspergillus fumigatus, Aspergillus niger and Aspergillus flavus* are common molds to which humans are exposed. Due to the increased use of immunosuppressant medications, the development of more intensive chemotherapies and the advent of AIDS, there has been in increase in the number of patients at risk of developing invasive aspergillosis. *Aspergillus* grows slowly, and can manifest in a variety of ways including chronic sinusitis and brain lesions. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Penicillium. *Penicillium* is a genus of fungi, which commonly grows on many foodstuffs such as cocoa beans, coffee beans, cassava flour, cereals, fish, peanuts, dried fruits, wine, poultry eggs and milk. In general, *Penicillium* species have low pathogenicity and infection is usually only seen in immunocompromised individuals, however cases have been reported without immune suppression. The clinical presentation of disease caused by *Penicillium* is diverse, and it is known to cause pathology via direct infection, allergic reaction, and pulmonary fibrosis. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Stachybotrys chartarum. *Stachybotrys chartarum* (*S. chartarum*) is a black mold that produces asexual spores. *S. chartarum* is the usual perpetrator involved in water/moisture/wet-damaged building illnesses. Invasion can occur via direct contact through the skin, or inhalation. Skin contact may result in dermatitis. Pulmonary exposure can result in rhinitis, cough, sore throat and chest tightness. *Stachybotrys* can release potent mycotoxins, which, if ingested, may contribute to systemic inflammation and disease pathogenesis. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

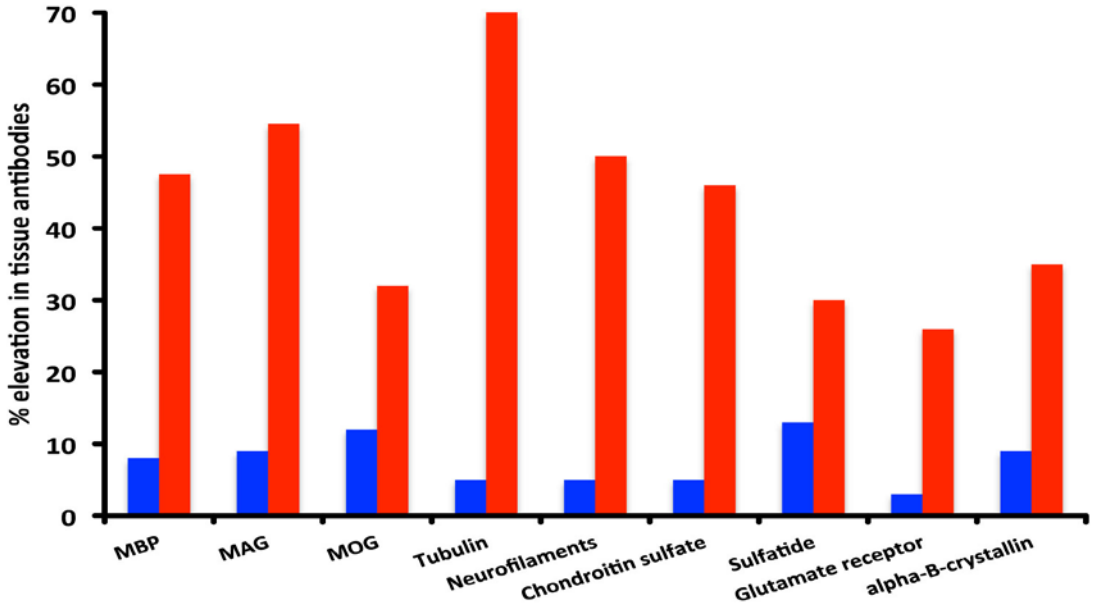

Figure 28. Percentage of elevation in antibodies against different neural antigens in healthy controls ■ and in patients exposed to molds ■. *Modified from Campbell A et al., Neural autoantibodies and neurophysiologic abnormalities in patients exposed to molds in water-damaged buildings. Arch. Environ. Health, 58(8):464-474, 2003.*

Citrullinated EBV. Epstein-Barr virus (EBV), or herpes type IV, is a DNA virus composed of linear double stranded DNA genome enclosed by a capsid and membrane derived envelope made from a variety of glycoproteins. EBV is ubiquitous. Infection often occurs during childhood and infects about 95% of the population. In adolescence, infectious mononucleosis, the "kissing disease," can occur, during which up to 20% of B-cells become infected with the virus. Citrullination or deimination is the conversion of the amino acid arginine in a protein into the amino acid citrulline. Citrullination of EBV occurs when citrullin, an amino acid that is created by post translational modification of arginine residues in proteins, is catalyzed by an enzyme called peptidyl arginine deiminase (PAD). Citrullinated EBV could act as a target for anti-citrullinated protein antibodies (ACPAs), autoantibodies that are directed against peptides and proteins that are citrullinated, and this characteristic may account for the link between EBV infection and the onset or progression of ACPAs production. Citrullination of a variety of proteins is emerging as an essential component of inflammation in a variety of autoimmune diseases including neuroautoimmunity. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

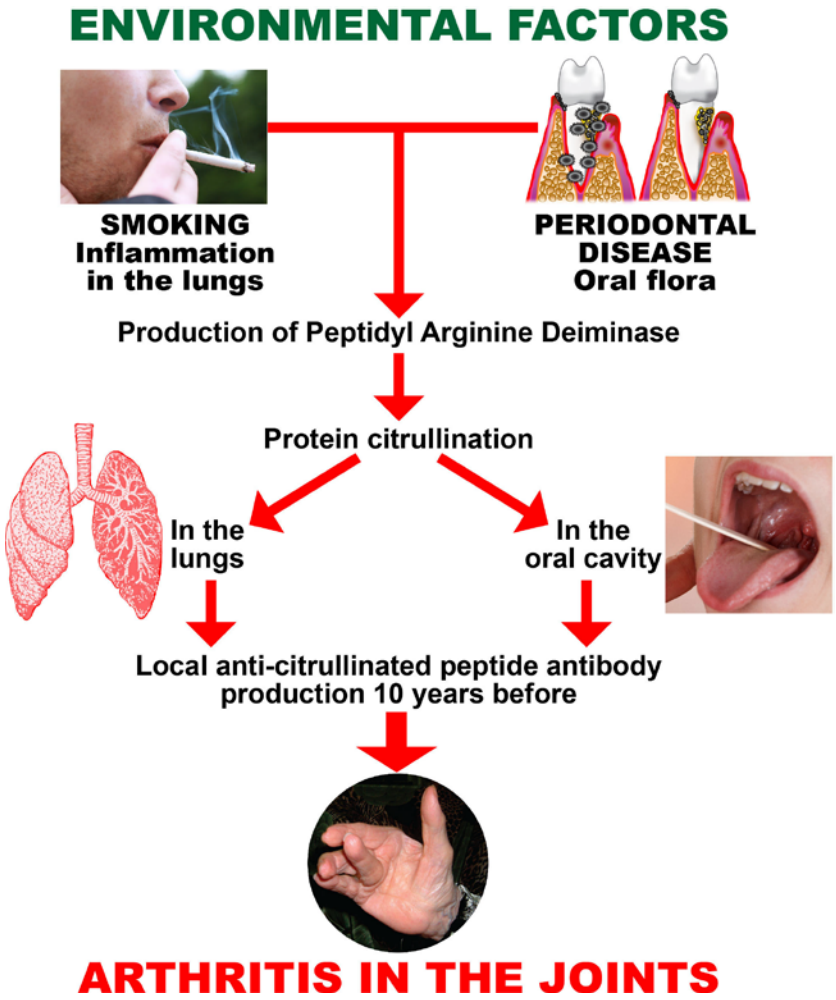

Figure 29. Mechanism of citrullination-induced autoimmunity. *Environmental triggers such as smoking or oral bacteria can cause the production of peptidyl arginine deiminase, which leads to protein citrullination in the lungs or in the oral cavity. The local anti-citrullinated peptide antibody builds over a period of years culminating in autoimmune reactivity against tissue.*

Hepatitis C virus. Hepatitis C virus (HCV) is an enveloped, positive-sense single-stranded RNA virus of the *Flaviviridae* family. Hepatitis C (HepC) or Non-A-, Non-B-Hepatitis, causes infection of the liver that is spread through the blood or body fluids. Using molecular mimicry, this virus is able to evade immune attack. Chronic Hepatitis C virus infection is known to induce autoimmune reactions and can be associated with extrahepatic manifestations including diabetes and thyroid autoimmune disorders. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

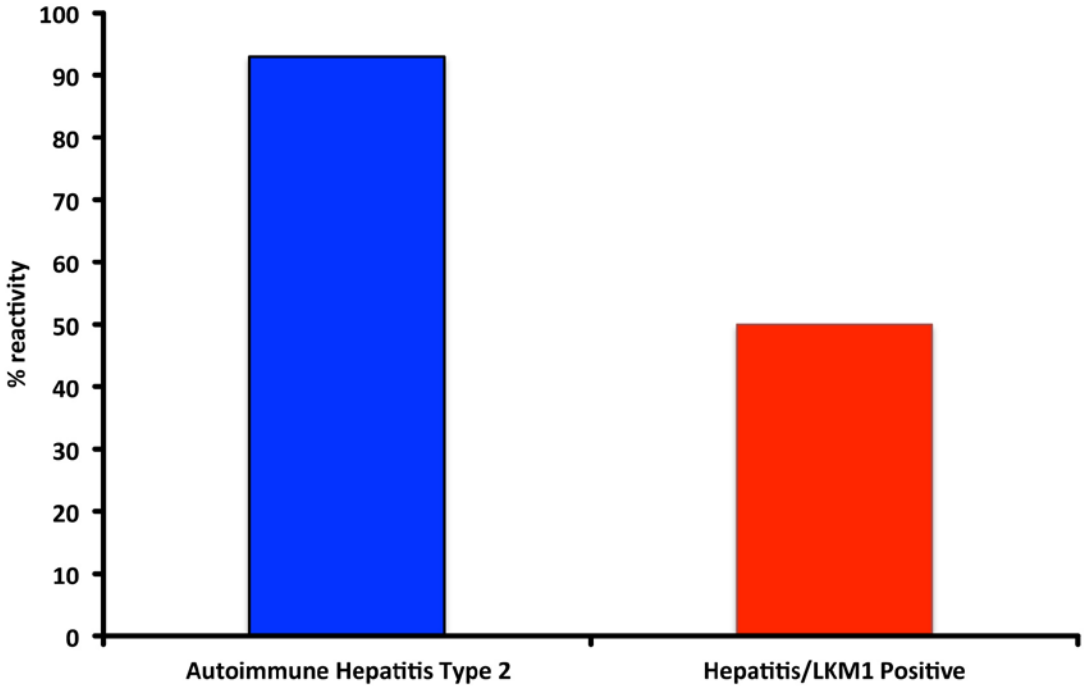

Figure 30. Percentage reaction of sera of patients with autoimmune hepatitis type 2 ■ and hepatitis/LKM$_1$ positive ■ patients with the immunodominant epitope of CYP2D6$_{193\text{-}212}$. From data in *Kerkar N et al., Cytochrome P4502D6$_{193\text{-}212}$: a new immunodominant epitope and target of virus/self cross-reactivity in liver kidney microsomal autoantibody type 1-positive liver disease. J Immunol; 170:1481-1489, 2003.*

Cytomegalovirus. *Cytomegalovirus* (CMV) is an opportunistic herpesvirus belonging to the *Betaherpesvirinae* subfamily, which is classified as herpes type-5. After primary infection, CMV can infect a variety of cell types such as epithelial cells of salivary glands, large intestine, lungs, smooth muscle, endothelial cells, liver, kidney, fibroblasts, neuronal cells and various myeloid cells. Persistent CMV has been implicated in systemic lupus erythematosus, multiple sclerosis, systemic sclerosis, type 1 diabetes and Sjögren's syndrome. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Figure 31. Sequence similarity between CMV and glutamic acid decarboxylase-65. *From data in Hiemstra HS et al., Cytomegalovirus in autoimmunity: T cell crossreactivity to viral antigen and autoantigen glutamic acid decarboxylase. PNAS; 98:3988-3991, 2001.*

Human herpesvirus-6. Human Herpesvirus-6 (HHV-6), belonging to the beta-herpesvirus subfamily, is a lymphotropic virus, which infects mainly T cells *in vitro*, causes acute and latent infections. Most humans acquire HHV-6 during early childhood and after initial infection, the virus latently remains in the host. HHV-6 is capable of persisting in the host after primary infection for many years. Salivary glands and brain tissue are suspected of harboring persistent HHV-6 infection. Candidate sites for latency are monocytes and early bone marrow progenitor cells. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

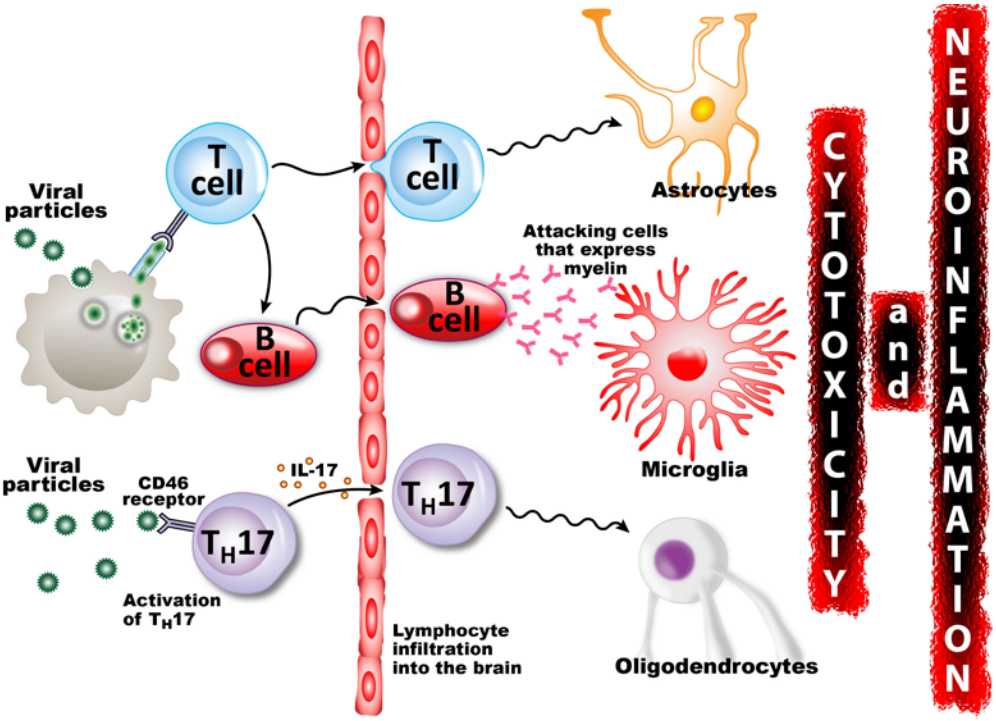

Figure 32. Mechanism by which HHV-6 induces neurotoxicity, neuroinflammation and neuroautoimmunity. *HHV-6 is capable of activating inflammatory immune responses that open the blood-brain barrier and trigger neuroautoimmunity.*

Borrelia burgdorferi. *Borrelia burgdorferi* is spirochete class bacterium. *B. burgdorferi sensu stricto, B. burgdorferi sensu lato, B. burgdorferi afzelii* and *B. burgdorferi garinii* spirochetes enter the human body through tick bites. Mixed with tick saliva, *Borrelia* travels through the circulation and enters different tissues. In some untreated cases, symptoms of pathogenic invasion have involved neurologic, cardiac, or joint disorders. *Borrelia* pathogenesis can break the blood-brain barrier, which allows invasion of the central nervous system, resulting in neuroborreliosis. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

Babesia + Ehrichia + Bartonella. *Babesia* is a tick-borne intraerythrocytic protozoan parasite, which can result in subclinical or mild illness in most cases, but occasionally, in immunocompromised individuals, the reaction can be severe. *Ehrlichia* is a tick-borne genus of rickettsiales bacteria, which targets neutrophils adherent to endothelium in tissues, resulting in tissue damage and inflammatory responses. *Bartonella henselae* is a tick-borne proteobacterium, which can present with nonspecific clinical features indicating upper respiratory tract infection or viral pneumonia. Please refer to corresponding white paper for details on infiltration, autoimmune mechanisms and references.

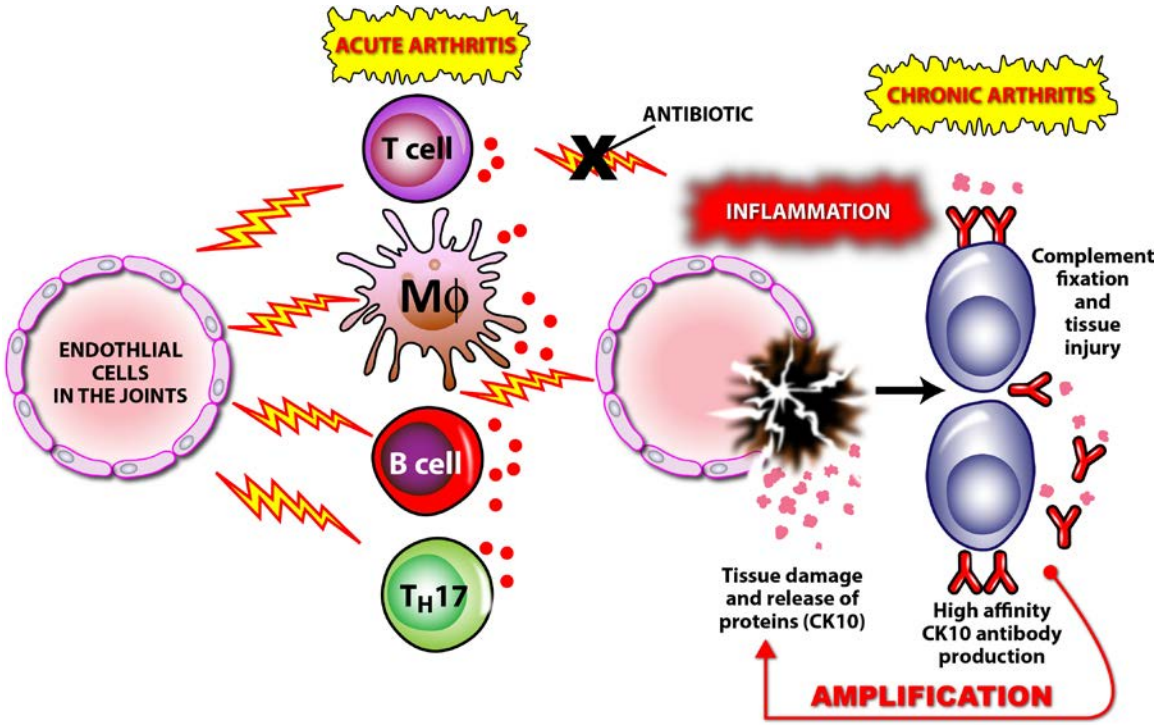

Figure 33. Induction of inflammation and tissue injury by *B. burgdorferi* and T, B cell response to CK10 in the inflamed synovial environment. *Tick-borne spirochetes are potent triggers of autoimmunity against joint tissues.*

Influencing Factors

The development of pathogen antigen antibodies depends not only on the amount and duration of the pathogen antigen exposure, but also on the genetic background,[44 45] diet[46 47 48 49] and lifestyle[50 51 52 53] of the person hosting the pathogen.[9]

Genetic

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 the heterogeneity of autoimmune prevalence. The importance of genes in autoimmunity was emphasized when it was noticed that the risk of autoimmunity is increased in twins and siblings of affected individuals.[54] Thereafter, gene analysis studies have confirmed the genetic relevance and suggested different methods for predicting the development of autoimmune conditions such as SLE, RA, DM1, and MS on an individual basis.[55 56 57] Immune system genetic variations that influence and imbalance the proinflammatory and anti-inflammatory immune responses can predispose the human to pathogen invasion. When the human immune response is off balance, it cannot adequately fight invading pathogens and thus, the individual may harbor multiple pathogens, which can ultimately lead to autoimmune reactivity. Furthermore, single-nucleotide polymorphisms (SNPs) that mediate susceptibility of humans to pathogens have been identified, allowing for identification of at risk individuals and, therefore, the prevention of infection.[45]

Environmental Exposures

The development of an autoimmune disease may be influenced by the genes a person inherits together with the way the person's immune system responds to certain environmental triggers, such as infectious agents and toxic chemicals. The role of environmental factors can be better understood when one considers that 1) only 24-50% of identical twins develop the same autoimmune disease and 2) the fact that major histocompatibility complex (MHC) differences are not the only factor contributing to the susceptibility to autoimmunity, but lifestyle, pathogen virulence factors, and exposure rates are important factors that cause individuals to react differently to the same pathogens.

***Clinical manifestations of pathogen exposures can present at any age*.** It is important to consider:

- Dietary risk factors
    - High sugar intake
    - High salt intake
    - Low protein intake
    - Drinking unfiltered water
- Location risk factors
    - Rural
        - Increased exposure to animal-borne pathogens
            - *Blastocystis hominis*
            - *Campylobacter jejuni*
            - *Klebsiella*
            - *Yersinia enterocolitica*
        - Increased exposure to soil-borne pathogens

- *Entamoeba histolytica*
- *Klebsiella*
- *Mycobacterium avium*

- Northeast and Upper Midwest regions – increased exposure to tick-borne pathogens
  - *Borrelia burgdorferi*
  - *Babesia*
  - *Ehrlichia*
  - *Bartonella*

- Urban
  - Increased exposure to communicable pathogens
    - Chlamydias
    - Epstein-Barr virus
    - *Cytomegalovirus*
    - *Helicobacter pylori*
    - Human herpesvirus-6
    - *Klebsiella*
    - Mycoplasmas
- Water
  - Untreated water such as lakes and ponds as well as unclean pool water
    - *Cryptosporidium*
    - *Entamoeba histolytica*
    - *Giardia lamblia*
    - *Klebsiella*
    - *Mycobacterium avium*
    - *Yersinia enterocolitica*
- Hospital
  - Increased exposure to hospital-borne infections
    - *Clostridium difficile*
    - *Hepatitis C virus*

- Stress level
  - Chronic stress
    - Reduces cortisol production
    - Alters intestinal microbiota
    - Opens tight junctions of essential body barriers
  - Acute stress
    - Opens tight junctions of essential body barriers

Array 12 can be used to:

- Detect immune reaction to key pathogens that may lead to multiple autoimmune reactivities.
- Determine the role of pathogens in cases of ‘unexplained’ autoimmune reactivities.

- Monitor the effectiveness of clinical protocols for addressing pathogens associated with multiple autoimmunities.

Array 12 is recommended for patients who:

- Present with chronic conditions such as gastrointestinal distress, fatigue, body aches or unexplained and general inflammation, including neuroinflammation.
- May have been exposed to bacteria, viruses, parasites, molds, and spirochetes associated with multiple autoimmunities.
- Test negative for dietary proteins and tissue-bound chemical antibodies, yet continue to present symptoms associated with autoimmunities.
- Have not fully responded to clinical interventions, such as detoxification and dietary modifications.

CLINICAL INTERPRETATION FOR ANTIBODY ARRAY 12 – PATHOGEN-ASSOCIATED IMMUNE REACTIVITY SCREEN

Elevated antibodies to pathogen antigens indicate immune reactivity to a pathogen remaining within host tissues.

Array 12 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.

Oral Pathogens

PATHOGEN ANTIGEN	CLINICAL SIGNIFICANCE
Porphyromonas gingivalis	Increased risk of rheumatoid arthritis
Streptococcus mutans	Increased risk of autoimmune cardiovascular disorders

Gastrointestinal Pathogens

PATHOGEN ANTIGEN	CLINICAL SIGNIFICANCE
Helicobacter pylori	Increased risk of gastrointestinal disorders, neurological disorders, rheumatic diseases, thyroid autoimmunity and lupus
Campylobacter jejuni	Increased risk of bowel disorders, neurological disorders and arthritis
Yersinia enterocolitica	Increased risk of gastrointestinal disorders, eye inflammation, thyroid autoimmunity, reactive arthritis
Clostridium difficile	Increased risk of gastrointestinal disorders including irritable bowels, ulcerative colitis and Crohn's disease
Candida albicans	Increased risk of gastrointestinal disorders and extra-intestinal autoimmunities
Rotavirus	Increased risk of gastrointestinal disorders, type 1 diabetes, eye autoimmunity

Gastrointestinal Parasites

PATHOGEN ANTIGEN	CLINICAL SIGNIFICANCE
Entamoeba histolytica	Increased risk of bone and neurological disorders
Giardia lamblia	Increased risk of gastrointestinal disorders including intestinal permeability and autoimmunity against gastrointestinal tract tissues
Cryptosporidium parvum	Increased risk of colon autoimmunity, Celiac disease and non-celiac gluten sensitivity
Blastocystis hominis	Increased risk of irritable bowel disorders and fibromyalgia

Bacterial and Stealth Pathogens

PATHOGEN ANTIGEN	CLINICAL SIGNIFICANCE
Human HSP-60 + Chlamydia HSP-60	Increased risk of multiple autoimmunities including arthritis, lupus, gastrointestinal disorders, lung disorders, heart autoimmunity, and neuroautoimmunity
Chlamydias	Increased risk of neuroautoimmunities, systemic inflammation, and autoimmune cardiovascular disorders
Streptozymes	Increased risk of neurological disorders including obsessive compulsive disorder (OCD), pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), rheumatic heart disorders and reactive arthritis
Streptococcal M Protein	Increased risk of neurological disorders including obsessive compulsive disorder (OCD), pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), rheumatic heart disorders and reactive arthritis
Mycoplasmas	Increased risk of lupus, arthritis and anti-phospholipid syndrome
Acinetobacter	Increased risk of multiple sclerosis
Klebsiella	Increased risk of joint, skeletal and eye autoimmunities
Mycobacterium avium	Increased risk of gastrointestinal disorders, thyroid autoimmunity, type 1 diabetes, arthritis and multiple sclerosis

Environmental Molds

PATHOGEN ANTIGEN	CLINICAL SIGNIFICANCE
Aspergillus	Increased risk of chronic fatigue syndrome, fibromyalgia, multiple autoimmunities including neuroautoimmunity
Penicillium	Increased risk of chronic fatigue syndrome, fibromyalgia, multiple autoimmunities including neuroautoimmunity
Stachybotrys chartarum	Increased risk of chronic fatigue syndrome, fibromyalgia, multiple autoimmunities including neuroautoimmunity

Viruses

PATHOGEN ANTIGEN	CLINICAL SIGNIFICANCE
Citrullinated EBV	Increased risk of multiple autoimmunities including joint, lupus, neurological, thyroid, and liver, and type 1 diabetes and multiple food immune reactivity
Hepatitis C virus	Increased risk of liver autoimmunity
Cytomegalovirus	Increased risk of type 1 diabetes, arthritis, lupus and neurological disorders
Human Herpesvirus-6	Increased risk of chronic fatigue syndrome, fibromyalgia, lupus, and autoimmunities of the nervous system, joints and thyroid

Tick-Borne Pathogens

PATHOGEN ANTIGEN	CLINICAL SIGNIFICANCE
Borrelia burgdorferi	Increased risk of blood-brain barrier damage, neurological disorders and arthritis
Babesia + Ehrlichia + Bartonella	Increased risk of blood-brain barrier damage, neurological disorders and arthritis

Array 12 is one of the Environmental Triggers assessments available through the Cyrex System™. Depending on the result of Array 12, it may be logical to identify Increased Barrier Permeability (Arrays 2 and 20), or to assess Biomarkers of Autoimmune Reactivity (Arrays 5, 6, 7/7X). **Table 2** is a guide to potential follow up testing after Array 12.

Table 2. Suggested follow up testing. Based on Array 12 results and additional pertinent clinical data, these are suggested follow up testing for Array 12.

Positive Result	Consider Follow Up Array					
	2	5	6	7/7X	8	20
Porphyromonas gingivalis	X	X			X	
Streptococcus mutans	X	X				
Helicobacter pylori	X	X	X			
Campylobacter jejuni	X	X		X		X
Yersinia enterocolitica	X	X			X	
Clostridium difficile	X	X				
Candida albicans	X	X	X	X	X	X
Rotavirus	X	X	X			
Entamoeba histolytica	X	X				
Giardia lamblia	X	X			X	
Cryptosporidium	X	X				
Blastocystis hominis	X	X				
Human HSP-60 + Chlamydia HSP-60	X	X				
Chlamydias	X	X			X	
Streptozymes	X	X		X	X	X
Streptococcal M Protein	X	X		X		X
Mycoplasmas	X	X			X	
Acinetobacter	X	X		X		X
Klebsiella	X	X				
Mycobacterium avium	X	X		X		X
Aspergillus	X	X		X		X
Penicillium	X	X		X		X
Stachybotrys chartarum	X	X		X		X
Citrullinated EBV	X	X	X	X	X	X
Hepatitis C virus		X				
Cytomegalovirus	X	X		X		X
Human Herpesvirus-6	X	X		X		X
Borrelia burgdorferi	X	X		X	X	X
Babesia + Ehrlichia + Bartonella	X	X				

SPECIMEN REQUIREMENT

2 mL serum
Ambient

RELATED TESTING

- **Antibody Array 2 - Intestinal Antigenic Permeability Screen**
- **Antibody Array 5 – Systemic Autoimmune Reactivity Screen**
- **Antibody Array 6 - Diabetes Autoimmune Reactivity Screen**
- **Antibody Array 7/7X – Neurological Autoimmunity Reactivity Screen**
- **Antibody Array 8 – Joint Autoimmune Reactivity Screen**
- **Antibody Array 20 – Blood Brain Barrier Permeability**

REFERENCES

1. Rybicki E. Where did viruses come from? Scientific American, 2008. Accessed 7/25/16 http://www.scientificamerican.com/article/experts-where-did-viruses-come-fr/
2. Bortman H. Tracking viruses back in time. Astrobiology Magazine, 2010. Accessed 7/25/16 http://www.astrobio.net/topic/origins/extreme-life/tracking-viruses-back-in-time/
3. Koonin EV, Senkevich TG, Dolja VV. The ancient virus world and evolution of cells. Biol Direct, 2006; 1:29.
4. Savage DC. Microbiol ecology of the gastrointestinal tract. Annu Rev Microbiol, 1977; 31:107-133.
5. Tojo R, Suárez A, Clamente MG, et al. Intestinal microbiota in health and disease: role of bifidobacteria in gut homeostasis. World J Gastroenterol, 2014; 20(41):15163-15176.
6. Sekirov I, Russell SL, Antunes LC, Finlay BB. Gut Microbiota in health and disease. Physiol Rev, 2010; 90(3):859-904.
7. Lebba V, nicoletti M, Schippa S. Gut microbiota and the immune system: an intimate partnership in health and disease. Int J Immunopathol Pharmacol, 2012; 25(4):823-833.
8. Kort R Caspers M, van de Graaf A, et al. Shaping the oral microbiota through intimate kissing. Microbiome, 2014; 2:41.

9. Christen U and von Harrath MG. Infections and autoimmunity – good or bad? J Immunol, 2005; 174:7481-7486.

10. Delogu LG, Deidda S, Delitala G, Manetti R. Infectious diseases and autoimmunity. J Infect Dev Ctries, 2011; 5(10):679-687.

11. Kivity S, Agmon-Levin N, Blank M, Shoenfeld Y. Infections and autoimmunity—friends or foes? Trends Immunol, 2009; 30(8):409-414.

12. Oldstone MBA, Nerenberg M, Southern P, et al. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell, 1991; 65(2):319-331.

13. Posnett DN and Yarilin D. Amplification of autoimmune disease by infection. Arthritis Res Ther, 2005; 7(2):74-84.

14. Faé KC, Da Silva DD, Oshiro SE, et al. Mimicry in recognition of cardiac myosin peptides by heart-intralesional T cell clones from rheumatic heart disease. J Immunol, 2006; 176(9):5662-5670.

15. Kumar D, Gemayel NS, Deapen D, et al. North-American twins with IDDM. Genetic, etiological, and clinical significance of disease concordance according to age, zygosity, and the interval after diagnosis in first twin. Diabetes, 1993; 42(9):1351-1363.

16. Yu L, Robles DT, Abiru N, et al. Early expression of antiinsulin autoantibodies of humans and the NOD mouse: evidence for early determination of subsequent diabetes. Proc Natl Acad Sci U S A, 2000; 97(4):1701-1706.

17. Notkins AL. New predictors of disease. Sci Am, 2007; 296(3):72-79.

18. Gamble DR, Kinsley ML, FitzGerald MG, et al. Viral antibodies in diabetes mellitus. Br Med J, 1969; 3(671):627-630.

19. Kaufman DL, Erlander MG, Clare-Salzler M, et al. Autoimmunity to two forms of glutamate decarboxylase in insulin-dependent diabetes mellitus. J Clin Invest, 1992; 89(1):283-292.

20. Sadeharju K, Lönnrot M, Kimpimäki T, et al. Enterovirus antibody levels during the first two years of life in prediabetic autoantibody-positive children. Diabetologia, 2001; 44(7):818-823.

21. Calcinaro F, Dionisi S, Marinaro M, et al. Oral probiotic administration induces interleukin-10 production and prevents spontaneous autoimmune diabetes in the non-obese diabetic mouse. Diabetologia, 2005; 48(8):1565-1575.

22. Vaarala O, Atkinson MA, Neu J. The “perfect storm” for type 1 diabetes: the complex interplay between intestinal microbiota, gut permeability, and mucosal immunity. Diabetes, 2008; 57(10):2555-2562.

23. Sechi LA, Rosu V, Pacifico A, et al. Humoral immune responses of type 1 diabetes patients to *Mycobacterium avium* subsp. *paratuberculosis* lend support to the infectious trigger hypothesis. Clin Vaccine Immunol, 2008; 15(2):320-326.

24. Kuchroo VK, Ohashi PS, Sartor RB, Vinuesa CG. Dysregulation of immune homeostasis in autoimmune diseases. Nat Med, 2012; 18(1):42-47.

25. Bayry J, Sibéril S, Triebel F, et al. Rescuing CD4+CD25+ regulatory T-cell functions in rheumatoid arthritis by cytokine-targeted monoclonal antibody therapy. Drug Discovery Today, 2007; 12(13-14):548-552.

26. Linterman MA, Rigby RJ, Wong RK, et al. Follicular helper T cells are required for systemic autoimmunity. J Exp Med, 2009; 206(3):561-576.

27. Simpson N, Gatenby PA, Wilson A. et al. Expansion of circulating T cells resembling follicular helper T cells is a fixed phenotype that identifies a subset of severe systemic lupus erythematosus. Arthritis Rheum, 2010; 62(1):234-244.

28. Jorgensen JL, Reay PA, Ehrich EW, Davis MM. Molecular components of T-cell recognition. Annu Rev Immunol, 1992; 10:835-873.

29. Singh H, Ansari HR, Raghava GP. Improved method for linear B-cell epitope prediction using antigen's primary sequence. PLoS One, 2013; 8(5):e62216.

30. Ray S, Sonthalia N, Kundu S, et al. Autoimmune disorders: an overview of molecular and cellular basis in today's perspective. J Clin Cell Immunol, 2012; S10:003.

31. Bangs SC, Baban D, Cattan HJ, et al. Human CD+ memory T cells are preferential targets for bystander activation and apoptosis. J Immunol, 2009; 182(4):1962-1971.

32. Suwannasaen D, Romphruk A, Leelayuwat C, Lertmemongkolchai G. Bystander T cells in human immune responses to dengue antigens. BMC Immunol, 2010; 11:47.

33. Brocke S, Gaur A, Piercy C, et al. Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. Nature, 1993; 365:642-644.

34. Cole BC and Griffiths MM. Triggering and exacerbation of autoimmune arthritis by the *Mycoplasma arthritidis* superantigen MAM. Arthritis Rheum, 1993; 36:994-1002.

35. Dalwadi H, Wei B, Kronenberg M, et al. The Crohn's disease-associated bacterial protein I2 is a novel enteric T cell superantigen. Immunity, 2001; 15:149-158.

36. Wucherpfennig KW. Mechanisms for the induction of autoimmunity by infectious agents. J Clin Invest, 2001; 108(8):1097-1104.

37. Munz C, Lunemann JD, Getts MT, et al. Antiviral immune responses: triggers of or triggered by autoimmunity? Nat Rev Immunol, 2009; 9:246-228.

38. Lehmann PV, Forsthuber T, Miller A, Sercarz EE. Spreading of T-cell autoimmunity to cryptic determinants of an autoantigen. Nature, 1992; 358:155-157.

39. Kaufman DL, Clare-Salzler M, Tian J, et al. Spontaneous loss of T-cell tolerance to glutamic acid decarboxylase in murine insulin-dependent diabetes. Nature, 1993; 366:69-72.

40. Cross AH, Tuohy VK, Raine CS. Development of reactivity to new myelin antigens during chronic relapsing autoimmune demyelination. Cell Immunol, 1993; 146:261-269.

41. Monneaux F and Muller S. Epitope spreading in systemic lupus erythematosus: identification of triggering peptide sequences. Arthritis Rheum, 2002; 46(6):1430-1438.

42. Hall MD and Ebert D. The genetics of infectious disease susceptibility: has the evidence for epistasis been over estimated? BMC Biol, 2013; 11:79.

43. Agmon-Levin N, Ram M, Barzilai O, et al. Prevalence of hepatitis C serum antibody in autoimmune diseases. J Autoimmunity, 2009; 32(3-4):261-266.

44. Pana Z-D, Farmaki E, Roilides E. Host genetics and opportunistic fungal infections. Clin Microbiol Infect, 2014; 20:1254-1264.

45. Frodsham AJ and Hill AVS. Genetics of infectious diseases. Human Mol Gen, 2004; 13(2):R187-R194.

46. Gunsalus KT, Tornberg-Belanger SN, Matthan NR, et al. Manipulation of host diet to reduce gastrointestinal colonization by the opportunistic pathogen *Candida albicans*. mSphere, 2015; 1(1). pii: e00020-15.

47. Katona P and Katona-Apte J. The interaction between nutrition and infection. Clin Infect Dis, 2008; 46(10):1582-1588.

48. Ooi JH, Waddell A, Lin YD, et al. Dominant effects of the diet on the microbiome and the local and systemic immune response in mice. PLoS One, 2014; 9(1):e86366.

49. Halliez MC and Buret AB. Extra-intestinal and long term consequences of *Giardia duodenalis* infections. World J Gastroenterol, 2013; 19(47):8974-8985.

50. Salleh MR. Life event, stress and illness. Malays J Med Sci, 2008; 14(4):9-18.

51. Segerstrom SC and Miller GE. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychol Bull, 2004; 130(4):601-630.

52. Schneiderman N, Ironson G, Siegel SD. Stress and health: psychological, behavioral, and biological determinants. Annu Rev Clin Psychol, 2005; 1:607-628.

53. Maas K, Chan S, Parker J, et al. Cutting edge: molecular portrait of human autoimmune disease. J Immunol, 2002; 169(1):5-9.

54. Moore JH, Parker JS, Olsen NJ, Aune TM. Symbolic discriminant analysis of microarray data in autoimmune disease. Genet Epidemiol, 2002; 23(1):57-69.

55. Aune TM, Maas K, Moore JH, Olsen NJ. Gene expression profiles in human autoimmune disease. Curr Pharm Des, 2003; 9(23):1905-1917.

56. Aune TM, Parker JS, Maas K, et al. Co-localization of differentially expressed genes and shared susceptibility loci in human autoimmunity. Genet Epidemiol, 2004; 27(2):162-172.

57. Liu Z, Maas K, Aune TM. Comparison of differentially expressed genes in T lymphocytes between human autoimmune disease and murine models of autoimmune disease. Clin Immunol, 2004; 112(3):225-230.

