CLINICAL APPLICATION GUIDE

ARRAY 20

Array 20 – Antibody

Blood Brain Barrier Permeability Screen™

Cyrex™ Laboratories

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CLINICAL APPLICATION GUIDE TO BLOOD-BRAIN BARRIER PERMEABILITY SCREEN™

OVERVIEW

The blood brain barrier (BBB) is a physical barrier between the brain and the circulating blood, formed by the arrangement of endothelial cells and tight junctions that line the capillaries, which supply blood to the brain. It is a highly selective barrier that restricts the movement of all soluble proteins greater than 400 Da from the blood across to the brain. Acting like a filter, the BBB protects the brain from infections, the products of infections such as lipopolysaccharides (LPS), and toxic chemicals, *etc*., that circulate in the blood. The BBB naturally permits the passage of essential metabolites, small hydrophobic (lipid soluble) molecules like oxygen, carbon-dioxide, hormones, *etc*.

The Blood-Brain Barrier Structures

The blood-brain barrier is made up of endothelial cells, extensive tight junctions, the capillary basement membrane (BM), astrocyte end-feet ensheathing the vessels and pericytes (PCs) embedded within the BM (see **Figure 1**).

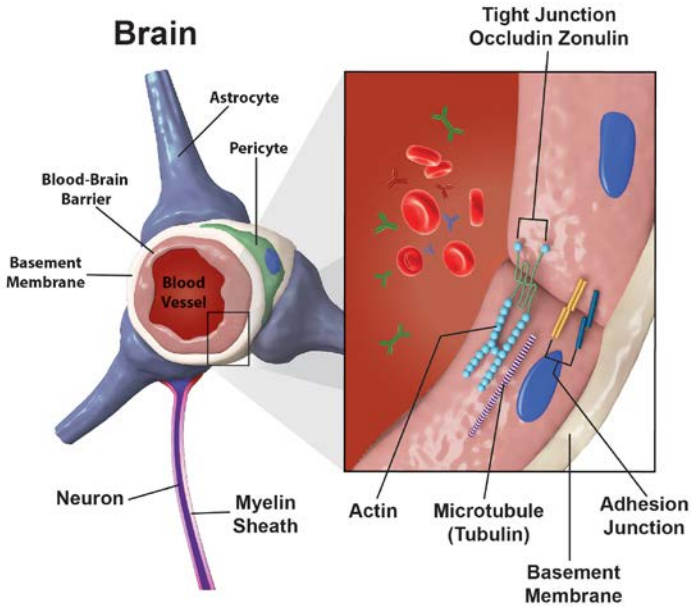

Figure 1. Structures of the BBB. The blood-brain barrier is a single layer of epithelial cells linked together by junction proteins.

BBB endothelial cells contain Actin, a smooth muscle protein forming a cable network within the endothelial cell, and Tubulin, a building block protein and a major component of a cell's internal cytoskeleton called microtubules.

Paracellular space between the endothelial cells of the BBB is protected by a unique, extensive network of tight junctions. BBB tight junctions consist of:

- Transmembrane proteins
  - Claudin
  - Occludin
  - Junction Adhesion Molecule (JAM)
- Cytoplasmic proteins
  - Zonulin-1 (ZO1)
  - Zonulin-2 (ZO2)
  - Zonulin-3 (ZO3)

Surrounding the BBB within the nervous system are pericytes (PCs) and astrocytic foot processes. Pericytes (PCs) are cells of microvessels such as capillaries, venules, and arterioles that wrap around the endothelial cells. PCs cover about 32% (about every 2 to 4 endothelial cells) of the area of BBB capillaries.[1] They are thought to provide structural support and vasodynamic capacity to the microvasculature.[2] In addition to PCs, the nervous system side of the BBB is also supported by astrocyte feet. More than 99% of the brain surface of the capillaries is supported by astrocytic foot processes.[3] Direct contact between endothelial cells and astrocytes is deemed necessary to generate optimal BBB function.[4] The basement membrane (BM) engulfs the BBB and PCs. It anchors the cells of the BBB in place and establishes the connection with the astrocytes and other resident cells.[5] This combination of structures is essential for proper BBB function.

Illuminating Point

The blood-brain barrier (BBB) is a single cell-layer structure that lines the capillaries reaching the nervous system and brain. It protects the brain and nervous system from infiltration by pathogens and other immunogens. Proteins making up the BBB are similar to the tissue proteins of the intestinal barrier.

The Blood-Brain Barrier Functions

The BBB is present in all brain regions, except in those regulating the autonomic nervous system and the endocrine glands.[1] In these areas, blood vessels permit diffusion of blood-borne molecules across the vessel wall.[6] Major BBB functions include:[2 7 8]

- maintenance of central nervous system (CNS) homeostasis
- protection of brain and nervous system from plasma components penetration
- specific transport of a constant supply of vital brain/CNS nutrients
- direct inflammatory cells to act in response to changes in local environment

The capillary system is the route for vital nutrients to reach the CNS, thus the number of capillaries in the CNS is believed be equal to the number of neurons. A working neuronal-vascular relationship is critical for optimal brain functioning. The brain receives up to 20% of cardiac out-put.[9] If blood flow to the CNS stops, brain functions stop in seconds and damage to neurons may occur in minutes.[10] The entire length of the extensive capillary network is lined with the BBB, which plays a role in health and disease processes.

Tight junctions between cells of the BBB endothelium form a physical barrier by significantly reducing passive diffusion through the paracellular pathway, which forces any molecular traffic to occur mainly across the endothelial cells, transcellular pathway.[11] Lipophilic molecules and gases such as oxygen and carbon dioxide can diffuse across the barriers via the lipid membranes. Specific solute carriers, or transporters, in apical and basolateral membranes, control the influx of small polar solutes needed by the brain (nutrients such as glucose and amino acids) and the efflux of many waste products.[11] Transporters also exclude many potentially toxic compounds, such as xenobiotics, viruses, food antigens and peptides present in the circulation.[11]

By extension, the BBB generally prevents developing B-cells from being exposed to unique brain antigens.[12] Therefore the immune system appears to have no mechanism for the establishment of tolerance to brain antigens or for the prevention of the production of antibodies against these antigens. Autoantibodies are known to appear in the bloodstream up to 10 years before the onset of some diseases such as diabetes, however in cases of neurological autoimmunity, the time frame is only up to 5 years.[13 14 15] With brain antigens being thus excluded it is increasingly likely that there could be an immune response to autoantigens such as myelin basic protein and other neural antigens during BBB breakdown.[16]

Illuminating Point

The immune system does not establish tolerance to brain antigens, thus, when there is a breakdown of the BBB, destruction of neurological tissues occurs quickly.

CLINICAL SIGNIFICANCE

The presence of BBB autoantigens may indicate a breakdown of the BBB. BBB protein autoantigens that have persisted for an extended period following repetitive BBB disruptions may cause neuronal cell death, the release of neural antigens, and an early cognitive decline. Traumatic brain injury (TBI) is a multifaceted pathology involving excitotoxicity, free radical formation, brain swelling, and the entry of locally produced molecules such as cytokines, chemokines, and other molecules (**Figure 2**).[17]

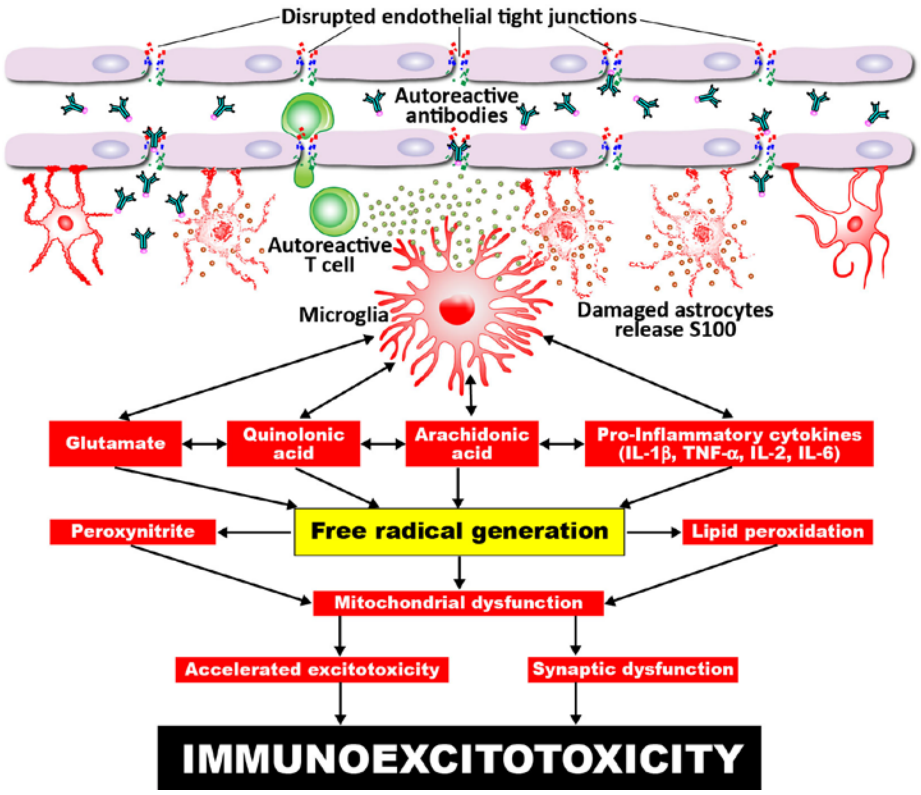

Figure 2. Proposed mechanism for excitotoxicity from leaky BBB. Repeated head trauma and multiple disruptions of the BBB leads to the release of free radicals and immunoinflammatory factors, causing acute microglia activation, which contributes to immunoexcitotoxicity.

For an excellent review on the role of the BBB in health and neurodegenerative disorders including Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis and Parkinson's disease, please see Zlokovic.[9] The target tissue damage is primarily determined by genetics and exposure to environmental factors, leading to various clinical conditions including:

- Neuroimmune disorders
- TBI[17]
- CNS involvement in systemic lupus erythematosus[18]
- Sarcoidosis[19]
- Autism spectrum[20]

The Central Role of BBB in Neuroautoimmunity

When the BBB is damaged it provides a gateway for environmental triggers to infiltrate the brain and nervous system. Due to the similarity between some of these triggers and neurological tissues, neuro-reactive antibodies can be formed. Neuronal autoantibodies contribute to the onset of neurological diseases. Known cross-reactions between neurological tissues and environmental triggers include:

- Asialoganglioside
  - Gliadin[21]
  - *Campylobactor jejuni* lipopolysaccharides[20]
  - Streptococcal proteins[22]
- Cerebellar
  - Gliadin[21 23]
  - Milk butyrophilin[23]
- Myelin Basic Protein
  - Gliadin[21]
  - *Chlamydia pneumoniae*[24]
  - Herpes-6[24]
  - Streptococcal protein[24 25]
- Myelin Oligodendrocyte Glycoprotein
  - Milk butyrophilin[23]
- Synapsin
  - Gliadin[21]
- Tubulin
  - Streptococcal protein[25]

Patients with circulating antibodies to the above environmental triggers who experience BBB breakage are at the risk of reactive antibodies infiltrating the brain and causing neurological tissue damage. The more circulating antibodies, the greater the potential damage to nervous system tissues can occur (see **Figure 3a** and **Figure 3b**). Once antibodies made against environmental triggers cause damage to these tissues, autoantibodies can be produced against the neurological tissues, which will further contribute to the neuroautoimmune process.

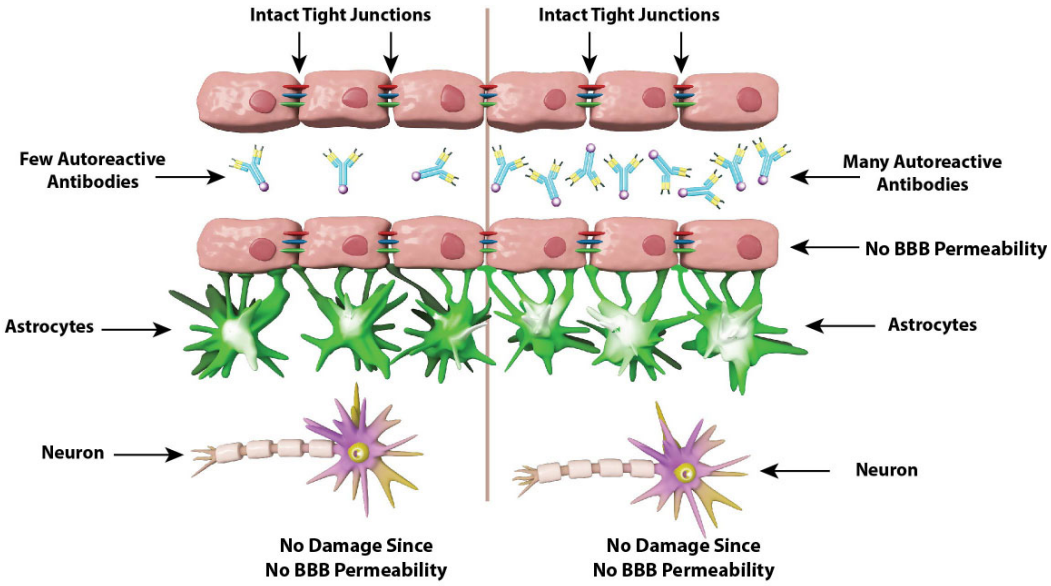

Figure 3a. Autoreactive antibodies and intact BBB. In an intact BBB, autoantibodies are restricted to the lumen of the blood vessels, so that the antibodies are prevented from causing damage to brain tissue, regardless of how high the antibody level is.

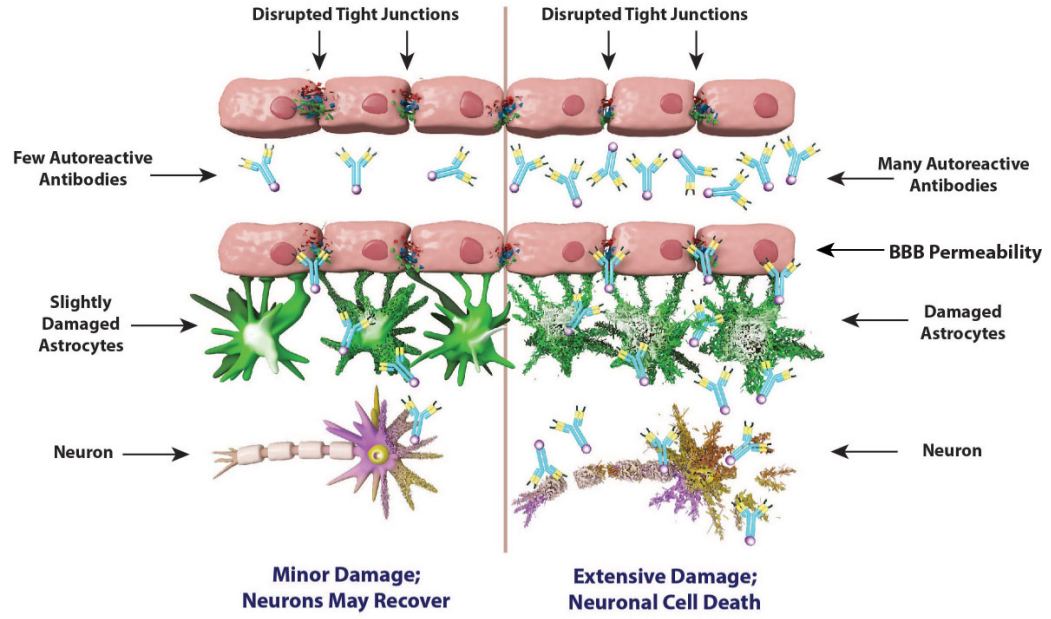

Figure 3b. Autoreactive antibodies and disrupted BBB. With a disrupted BBB, autoantibodies gain access to the brain tissues. With only a few autoreactive antibodies, damage is minimal and the neurons and brain may recover. However, a high level of autoreactive antibodies can lead to extensive neuronal damage, neuronal cell death, and possibly neuroautoimmune disorders.

Illuminating Point

When the BBB is intact, even multiple serological autoreactive antibodies cannot reach the brain, but when there is a breach of the BBB, the more autoreactive antibodies in circulation equates to greater risk of neurological tissue damage.

PATHOPHYSIOLOGY (MECHANISMS OF TISSUE DAMAGE)

Increasing data over recent years has linked the BBB and vasculature involvement in course of pathophysiology of many neurological issues. Inflammation induced by environmental triggers is a known cause of BBB disruption. Inflammatory disorders such as meningitis and encephalitis, and autoimmune diseases like multiple sclerosis, epilepsy and Alzheimer's disease may be initiated due to BBB breakdown.

Inflammation increases the permeability of brain capillary endothelial cells which let the immune cells such as neutrophils migrate into brain tissue.[6 11] When the BBB is inflamed, the tight junctions open and produce a condition called increased BBB permeability or "leaky brain." Xenobiotics, viruses and other molecules which are normally excluded can penetrate the BBB through tight junction openings and cause neuroautoimmunity with CNS symptoms.[26 27]

The repeated head trauma and TBI associated with some sports, such as football, have also been shown to induce BBB permeability, followed by antibody production against BBB proteins.[28] A cell-mediated response resulting in autoantibodies to BBB indicates a pathological alteration of the protective brain barrier.[29] As shown in Figure 2, such a result may manifest itself in pre- and post-synaptic dysregulation of neurotransmitters.[26 27]

The BBB works efficiently to prevent brain infections. However viruses can penetrate the barrier by attaching onto circulating cells of the immune system.[30] Systemic LPS enhances both immune cell[31] and free virus[32] transport across the intact BBB. LPS acts at the luminal surface of the brain microvascular endothelial cell monolayer, which induces abluminal secretion of cytokines and other factors that in turn act on pericytes; the pericytes then secrete substances that enhance viral transcytosis across the BBB.[33]

LPS is not the only molecule that has been shown to transport viruses across the BBB. In an animal study, wheat germ agglutinin (WGA), a lectin protein from wheat, was injected into the subjects. Banks and colleagues[34] showed by both *in vivo* and *in vitro* studies that free HIV-1 can be taken up by brain

endothelial cells and cross the BBB. When WGA binds to sialic acid and N-acetylglucosamine, it induces vesicle-mediated internalization of WGA by brain endothelial cells, a process called adsorptive endocytosis.[35 36] Lectin-induced vesicles provide the mechanism by which enveloped viruses in general are internalized by cells.[37] Banks' study results strongly suggest that glycoprotein gp120 or gp120/gp41 induces adsorptive endocytosis and the uptake of HIV-1 by brain endothelial cells.[34] This action explains how free, blood-borne viruses can infect the CNS while the BBB remains intact.

The three circumventricular organs of the brain (the subfornical organ, organum vasculosum of the lamina terminalis and the area postrema), as shown in **Figure 4**, is where the blood brain barrier is weak.[38] These regions allow a less restricted transport of molecules into the brain. At these regions neurons can be exposed to the environmental triggers that are found in the circulation.

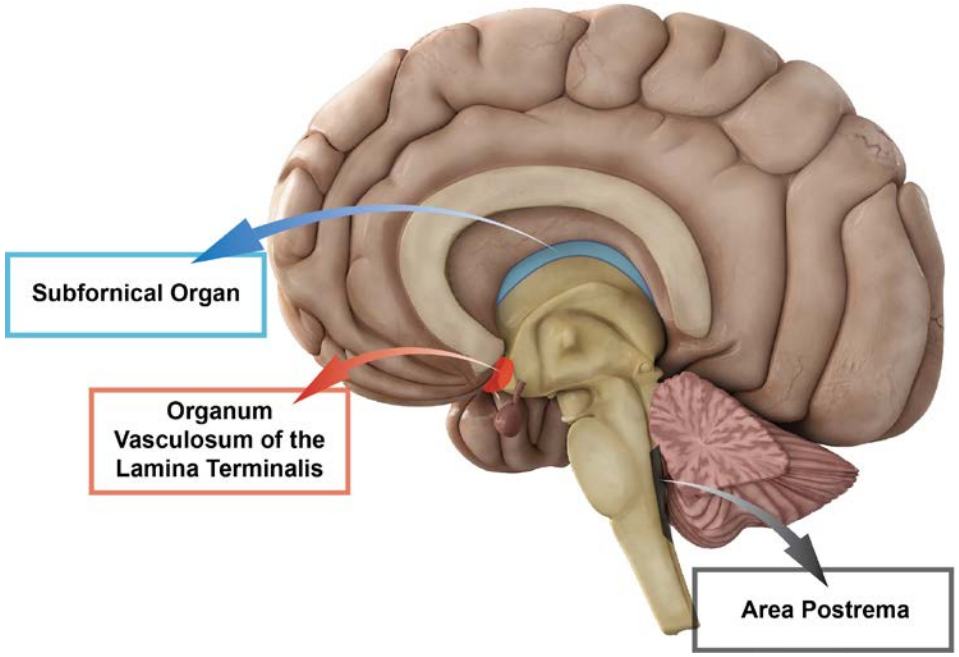

Figure 4. Three circumventricular organs of the brain. These are areas of the brain that are weakly protected.

BBB dysregulation plays a role in many neurological disorders. BBB breakdown may precede, accelerate, exacerbate or contribute to chronic disease processes in neurodegenerative disorders of the adult and aging nervous system.[9] Examples include, but are not limited to:[9]

- faulty BBB clearance of potential brain toxins in Alzheimer's disease and Parkinson's disease;
- inefficient clearance of excitotoxins across the BBB after an ischemic insult or TBI;
- increased transport of leukocytes across the activated BBB in multiple sclerosis, AIDS dementia, and Alzheimer's disease, and during neuroinflammatory CNS responses;
- BBB breakdown in amyotrophic lateral sclerosis, Alzheimer's disease, epilepsy and multiple sclerosis.

Illuminating Point

Breakdown in the BBB plays a role in multiple disorders including Alzheimer's, Parkinson's, traumatic brain injury, multiple sclerosis, AIDS dementia, amyotrophic lateral sclerosis and epilepsy.

INFLUENCING FACTORS

The triad concept of autoimmunity consists of three important components often present for the development of an autoimmune disease:

1. Genetic background
2. Environmental components
    a. Stress – both physical and emotional
    b. Chemicals and heavy metals (Array 11)
    c. Pathogens (Array 12)
    d. Dietary proteins and peptides (Arrays 3, 4, 10)
3. Barrier permeability
    a. Intestinal (Array 2)
    b. Blood-brain (Array 20)
    c. Skin
    d. Lung

In the clinical setting, review the patient's family history and/or test genetic markers to assess risk of developing BBB permeability. Include an investigation into environmental factors that includes the patient's current medical history in relationship to environmental exposures. Keep in mind that patients with a history of chronic increased intestinal permeability are at greater risk for developing increased BBB permeability.

Genetic

Although genetic and environmental factors both play a central role in neuroautoimmunity, many times it is not clear which one is the main link to heterogeneity of neuroautoimmune prevalence. The importance of genes in autoimmunity became emphasized when it was noticed that the risk of autoimmunity is increased in twins and siblings of affected individuals.[39] Gene analysis studies thereafter have confirmed the genetic relevance and suggested different methods for predicting the development of autoimmune conditions such as systemic lupus erythematosus, rheumatoid arthritis, diabetes mellitus type 1, and multiple sclerosis on an individual basis.[40 41 42 43]

A genetic brain disorder is caused by a variation or a mutation in one or more genes. A "variation" is a different form of a gene. A "mutation" is a change in a gene. Some genetic brain disorders are due to random gene mutations or mutations caused by environmental exposure to such xenobiotics as cigarette smoke, heavy metals, viruses or chemicals. Other disorders are strictly inherited, such as Huntington's disease, which means that a mutated gene or group of genes is passed down through a family. Other neurological disorders are rare inherited forms, such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis, which occur sporadically and frequently in human populations. Many neurological disorders can also be due to a combination of both genetic changes and environmental factors that affect gene expression.

An excellent review of the genetics of neurological diseases by Faghihi *et al*[44] outlines recent discoveries for the genes implicated in Alzheimer's disease, frontotemporal dementia, Pick's disease, normal pressure hydrocephalus, Parkinson's disease, amyotrophic lateral sclerosis and Kennedy's syndrome. The technologies born in the 1980s led scientists to begin mapping and identifying disease genes in the 1990s. Their work has advanced our understanding of the pathogenic basis of numerous neurological disorders. Genetic studies trying to elucidate Parkinson's disease for well over a decade have led to 28 distinct chromosomal regions more or less convincingly related to Parkinson's.[45] Only six of these specific regions contain genes with mutations that conclusively cause monogenic Parkinson's; mutations in these six genes explain only 3%– 5% of sporadic disease occurrences.[45] Clearly, the etiology of Parkinson's disease is multifactorial, with genes, environmental factors and the effect of environmental triggers on genes all taking a toll on the developing and aging brain.

Similarly, scientists have long been working on identifying genetic markers of Alzheimer's disease. Genome-wide association studies have been conducted around the world,[46] [47] [48] [49]

Environmental

A variety of environmental factors that can affect BBB permeability and initiate immunological inflammatory cascades. These include:

- Acute and repeated head trauma (both physical and emotional stressors)
- Stress
- Systemic LPS
- Chemical toxicity

Environmental factors are especially important in pregnant women since embryo BBB and endothelial barriers are immature and thus extremely vulnerable to be influenced by environmental toxins.[50]

MEDICAL HISTORY

Although specific genes variants and mutations have been identified, other factors have been shown to have greater impact on increasing a person's risk for developing Alzheimer's disease. According to the studies reviewed by Mayeux and Stern,[51] the following can be associated with and increased risk of Alzheimer's:

- Traumatic head injury
- Cardiovascular disease
- Smoking
- Type II diabetes
- Obesity

Although genes have been identified in Alzheimer's disease, outside factors have a great impact on disease development and risk.

Reviewing current medications, supplements, diets, and medical history may be critically important in determining who may have BBB permeability, or intestinal permeability which can lead to BBB permeability. The following factors should be considered:

- Playing contact sports in which repeated head trauma occurs
- Current or chronic viral infection
- History of chronic intestinal permeability and translocation of lipopolysaccharides
- History of systemic LPS
- Workplace and home exposures to xenobiotics
- Chemical intolerance leading to body burden
- Additional risk factors
    - Lack of exercise
    - Lack of relaxation

Illuminating Point

Due to cross-reactivity against actin, myosin, zonulin, claudins and other similar tissue proteins shared with the intestinal and blood-brain barriers, a broken intestinal barrier can lead to a breach in the BBB and vice versa.

MEASURING BLOOD-BRAIN BARRIER PERMEABILITY

By assessing serum antibodies to the protein structures that make up the blood-brain barrier, one can identify cases of BBB breakdown. When the BBB is damaged, circulating antibodies that cross-react with neurological tissues may infiltrate the nervous system and brain. The infiltration may lead to the destruction of neurological tissues and thus the production of autoantibodies to these neurological tissues. The cycle of neuroautoimmunity begins with a breach of the gut and blood-brain barriers.

CLINICAL USE OF CYREX ANTIBODY ARRAY 20

The gut-brain connection is well documented in the literature.[52 3 54 55] Due to antigen similarity between gut endothelium tissue proteins and BBB tissue proteins a correlation of "leaky gut" resulting in "leaky brain" can be made. Additionally, repeated or acute TBI can result in BBB dysregulation.

Array 20 may be used for:

- Early investigation for the management of contact sports-related TBI.
- Evaluation of the breach of the Blood Brain Barrier by stress, trauma or environmental triggers even in the absence of apparent concussion or brain injury.
- Assist in the assessment of increased risk of neurodegenerative disorders including, but not limited to multiple sclerosis, amyotrophic lateral sclerosis (Lou Gehrig's disease), senile dementia, Alzheimer's disease, Parkinson's disease, epilepsy and stroke.

Array 20 is recommended for patients who:

- Play contact sports during which repeated head trauma occurs
- Engage in activity that involves force or shock to the body and/or head.
- Have intestinal permeability with abnormal elevation in LPS and/or occludin/zonulin antibodies.
- Exhibit changes in cognitive function.

CLINICAL INTERPRETATION FOR ANTIBODY ARRAY 20

Interpretation of elevated level of antibodies against BBB Proteins is shown in **Table 1**.

Table 1. Interpretation of antibodies against BBB proteins assessed on Array 20.

Interpretation of Antibodies Against BBB Protein		
BBB IgA + IgG On-going reactivity	-	+
BBB IgM Early onset reactivity	-	+
Clinical Indication	No breakdown of BBB	Breakdown of BBB
Clinical Approach		Implement BBB repair protocols

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 7 – Neurological Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 7X – Expanded Neurological Autoimmune Reactivity Screen (Serum)**
- **Antibody Array 10 – Multiple Food Immune Reactivity Screen (Serum)**
- **Antibody Array 11 – Chemical Immune Reactivity Screen (Serum)**
- **Antibody Array 12 – Pathogen-Associated Immune Reactivity Screen (Serum)**

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