

## Association between environmental triggers and neuroautoimmunity in autism spectrum disorders

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### Abstract

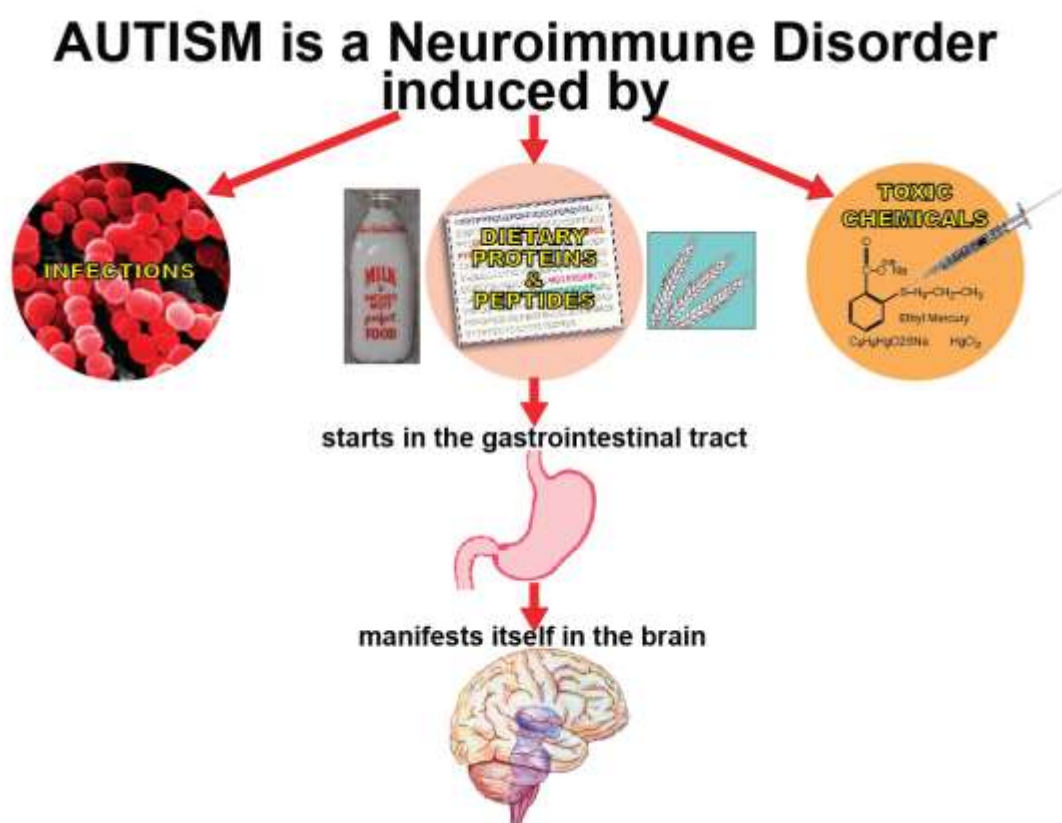
In this article, we aim to illustrate the various possible mechanisms that play a role in the multi-faceted neuroinflammation seen in Autism Spectrum Disorders, which involve the gastrointestinal, immune and nervous systems. As with other environmentally-induced autoimmune disorders, autism is a combination of genetic susceptibility, environmental triggers and barrier dysfunction. The pathogenesis of autism can take many avenues, from gut dysbiosis, to loss of intestinal barrier integrity, to systemic inflammation, to breach of the blood-brain barrier, to neuroinflammation and neuroautoimmunity. The gut-brain axis has been shown to play an important role in the induction of neuroautoimmune disorders. The connective inter-relation between gut and brain means that dysfunctions or damage to the intestinal barrier or blood-brain barrier can seriously affect one or the other. Environmental triggers actually begin their assault while someone is still in the womb; studies have shown that the efficiency of a person's immune system or his susceptibility to autoimmune disease can be affected by prenatal conditions, maternal exposures, and continuing exposure throughout a person's lifetime. Toxic chemicals abound in all aspects of existence, from food to medication to packaging to pollution. Individuals may be immune-reactive to particular chemicals bound to human tissue or food proteins. Infections can affect the immune system and breach the immune barriers. The triggering factors may also bind to human tissue, including neural tissue, causing tissue reactivity and neuroautoimmunity. By understanding the mechanisms by which these environmental triggers lead to neuroautoimmunity, clinicians may be able to identify the triggers, remove them from a patient's environment, and devise protocols to repair the barriers and improve the patient's health.

**Key words** Autism Spectrum Disorders, neuroinflammation, lipopolysaccharides, xenobiotics, body burden, intestinal permeability, blood-brain barrier

## 1. Introduction

Autism is a multifaceted disorder that involves the gastrointestinal (GI), immune and nervous systems, which mirrors many complex disorders.<sup>1</sup> Gene studies, using comparable methodologies, have been the focus of many researchers in the search for a cause of autism spectrum disorders (ASD). Others have looked at the environment as a trigger for ASD. Because those assessing the environment performed studies with various methodologies, a consensus on

environmental triggers has not been made.<sup>2,3</sup> Despite the debate, we believe both genetics and environmental factors, such as dietary components, toxic chemicals and infections, play a role in the development of autism. As early as 2005, the author of US patent 7,252,957 B2 postulated that autism is a neuroimmune disorder induced by environmental triggers.<sup>4</sup> The process begins in the GI tract, but manifests itself in the brain (Figure 1).<sup>5-7</sup>



**Figure 1. Induction of neuroimmune disorders by infections, toxic chemicals and dietary proteins or peptides in autism.**

We also believe that the heterogeneity of autism stems from a variation in the genetic makeup, and exposure to the environmental triggers during gestation and the first two years of life, in which both the immune system as well as the nervous system are in the process of maturation.<sup>8-14</sup> This may

include the father's lifestyle and the quality of sperm,<sup>15-16</sup> the mother's lifestyle and the quality of the egg,<sup>11-13</sup> gestational exposure to environmental toxins,<sup>10,17,18</sup> mode of birth,<sup>19,20</sup> breast feeding versus baby formula,<sup>21-23</sup> vaccinated versus unvaccinated,<sup>24-26</sup> time of solid food

introduction<sup>27,28</sup> and more to be elucidated by future research. All of these factors, or a combination of them, can affect the establishment of, and the integrity of gut microbiota, induction of oral tolerance, mucosal and overall immune function.<sup>29-35</sup>

Another crucial factor to consider is the actual role of the microbiome in autoimmune disorders. A very recent study points to the involvement of abnormal microbiota in the mechanisms that can lead to autoimmunity.<sup>36</sup> In a dysfunctional or imbalanced microbiome, harmful organisms may overwhelm beneficial or commensal bacteria. A deeper understanding of the role played by microbiota in autoimmune disorders can lead to a better understanding of the mechanisms of autism, and possibly to appropriate intervention protocols.

## 2. Environmental Triggers

The incidence of ASD is increasing worldwide, mainly in western countries. The National Health Statistics Report of 2015 in the USA reported 1 in 45 children 3-17 years of age is on the autism spectrum.<sup>37</sup> The role of the environment in ASD is becoming clearer.<sup>38</sup>

Toxic chemicals such as mercury, aluminum, formaldehyde, glutaraldehyde, bisphenol-A, artificial food coloring, in the environment and in food products, and vaccines or medications, have the capacity to break the oral tolerance mechanism and increase intestinal permeability. This breach in intestinal integrity not only results in the release of intestinal tight junction proteins, but also allows for the entry of unwanted molecules into the bloodstream. These immunogenic antigens such as dietary proteins, bacterial toxins, pathogenic components and xenobiotics can cause the activation of inflammatory and autoimmune cascades.<sup>36,39</sup> In relation to ASD and failure of oral tolerance, it was shown that perinatal exposure to food contaminants and

environmental toxins not only affects the mucosal, but also systemic, immune responses to the food antigens at adulthood.<sup>40-42</sup> Although Offit et al.<sup>43</sup> theorize the infant immune system can handle exposure to multiple, simultaneous antigens, others have shown that the naïve immune system of the neonate is more vulnerable to low doses of chemicals that trigger food immune reactivity and possibly autoimmunities later in life.<sup>39,40</sup>

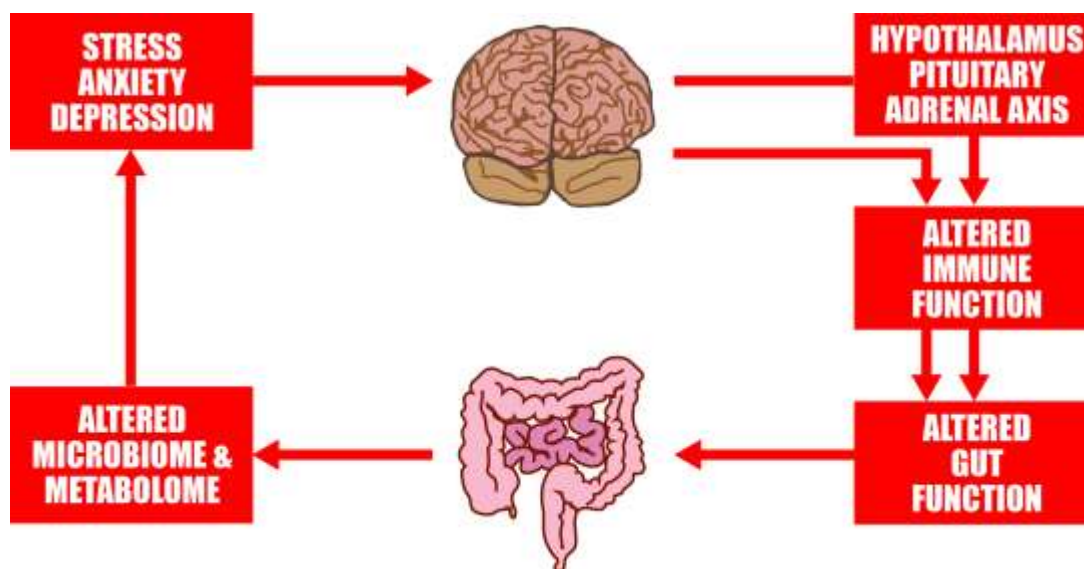
Environmental triggers include physical and emotional stress, dietary proteins and peptides, chemicals and heavy metals, and infectious agents. Working singly or in concert, environmental triggers can reach a threshold of burden, which may activate a cascade that manifests downstream as neuroautoimmunity, as seen in ASDs. Mechanisms of environmental triggers are outlined below.

### 2.1. Stress

Different kinds of stress can be connected to neuroautoimmunity in various ways. Chronic emotional stress or stressful events may result in chronic sympathetic nervous system (SNS) stimulation. During this stimulation there is an increase in blood pressure, heart rate and catecholamine secretions, causing an imbalance in epinephrine (adrenaline), norepinephrine (noradrenaline) and dopamine.<sup>44</sup> This disruption of the SNS in turn affects the functioning of the autonomic nervous system (ANS), affecting the hypothalamic-pituitary-adrenal (HPA) axis. The ANS and HPA play major roles in managing emotional stress.<sup>44</sup> Physical and emotional stress has been shown to open the tight junctions of the intestinal and blood-brain barriers.<sup>45</sup> In addition, chronic stress can alter the microenvironment for resident bacteria and contribute to gut dysbiosis. Gut dysbiosis leads to pathological changes in the epithelium, tight junctions and in the

capillary endothelium.<sup>46</sup> Psychological stress-induced intestinal permeability may be mediated through the parasympathetic nervous system, while physical stress, by way of prolonged strenuous exercise, and/or heat stress, contributes to increase intestinal

permeability through reductions in blood flow to the intestinal tissues that leads to oxidative stress.<sup>47</sup> This activation of the HPA axis by stress and its effect on the immune function and alteration of the gut microbiome is shown in Figure 2.



**Figure 2. The role of the microbiome and metabolome in stress, anxiety and depression.**

## 2.2. Dietary Proteins

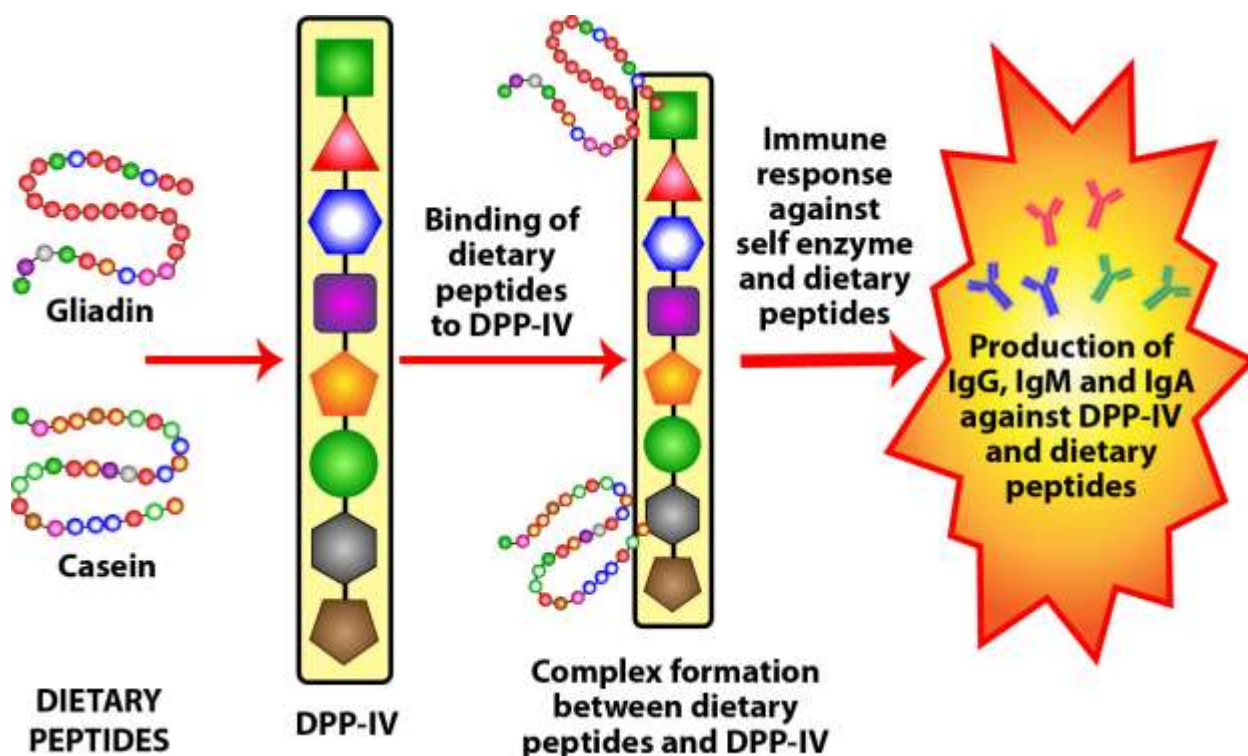
Many combinations of environmental factors can ignite gut inflammation and food immune reactivity.<sup>48</sup> Difficult to digest food antigens such as lectins, agglutinins, gliadins, gluteomorphins, caseins, casomorphins, milk butyrophilin, egg white and others are notorious instigators of gut dysfunction in ASD.<sup>6,49-50</sup> Food immune reaction to particular food proteins and peptides may be contributing to behavioral disorders in ASD. Among many food items, wheat and dairy studies prevail. It has been reported that the majority of children with autism cannot tolerate wheat or dairy products, and hence the elimination of these proteins and peptides from the diet is standard practice.<sup>50</sup> By avoiding wheat and dairy exposures, ASD conditions greatly improve.<sup>51-53</sup> The improvement in clinical conditions could be related to the cross-reactivity

between gliadin and cerebellar, and casein with myelin oligodendrocyte glycoprotein (MOG). Cross-reactivity can occur if amino acids sequences of the food protein and human tissue protein share similar structures. The antibodies made against the food protein may mistake the tissue for the food. Autoantibodies can then be formed and result in autoimmune reactivity to the tissue. By removing the environmental triggers, in this case, gluten and dairy, the result is lower cellular and humoral immune responses to neurological tissues.<sup>50,54</sup> By cutting off the source, neural tissue destruction can be arrested.

Another mechanism that could be responsible for the induction of autoimmunity in ASD is the binding of food generated peptides, such as gluten and casein, to tissue enzymes, such as dipeptidyl peptidase-4 (DPP-IV).<sup>6</sup> This mechanism induces autoimmunity against self-tissue

antigens and enzymes. In this case scenario, proper food digestion is disrupted, which leads to changes in gut microbiota and

eventual increased intestinal permeability (Figure 3).



**Figure 3. Dietary peptides binding to DPP-IV, formation of Hapten Carrier Effect, and production of antibodies against DPP-IV and dietary peptides.** This may result in dysfunction of the DPP-IV molecule and accumulation of peptides in the GI tract and in the circulation, leading to further immune response.

### 2.3. Chemicals/Heavy Metals

More than 7 million recognized chemicals are in existence, and approximately 80,000 of them are in common use worldwide, of which less than 5% have been studied for toxicity by the United States Environmental Protection Agency.<sup>55</sup> Some of these chemicals resist metabolism<sup>56-59</sup> and therefore accumulate in body tissues, which is referred to as the body burden of chemicals. Binding of xenobiotics or their metabolites with human tissue makes our body more likely to attack its own tissue by mistake.<sup>60,61</sup> Either through diet, lifestyle or

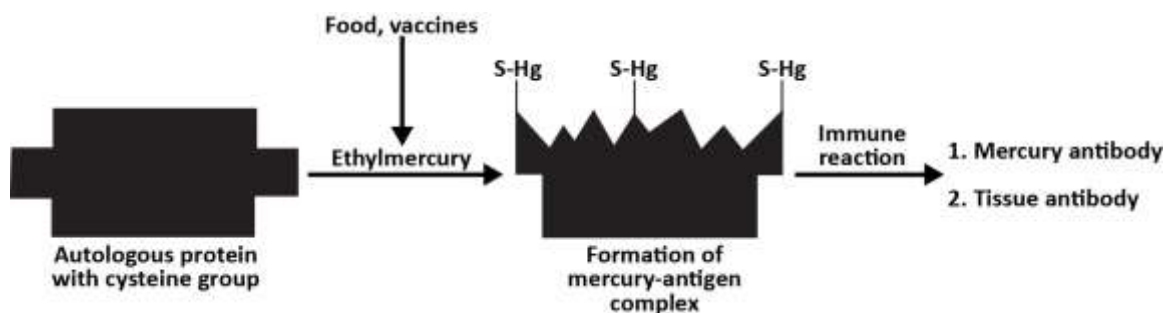
vaccination, nearly every individual is exposed to chemicals from birth and throughout his/her lifetime. Babies are born with multiple chemicals already accumulating in their bodies. In 2005, researchers took blood from umbilical cords of randomly selected individuals in US hospitals and tested it for 413 toxic chemicals and found 287 pesticides and pollutants in the cord blood.<sup>62</sup> Of the 287 chemicals detected, 180 are known to cause cancer, 217 are toxic to the brain and nervous system, and 208 are known to cause birth defects or abnormal development.



While resources limited the study to only 413 out of the known chemicals in use at the time, one can imagine how many more toxic chemicals would have been found in the cord blood.

**2.3.1. Mercury.** The extensive use of ethyl mercury (EtHg) as a preservative in vaccines from the 1930s until 1999, led to the belief that thimerosal could be linked to autism, autoimmunity and other conditions.<sup>63,64</sup> Thimerosal contains 49.6% Hg by weight and is metabolized to EtHg and thiosalicylate.<sup>65</sup> The normal dose of a pediatric vaccine contains about 12.5–25 µg of Hg per 0.5 ml.<sup>66</sup> Geier and Geier<sup>67</sup> compared thimerosal containing Diphtheria, Tetanus, and Pertussis (DTaP) vaccine cases to thimerosal-free DTaP vaccine cases and found a statistically significant increase in

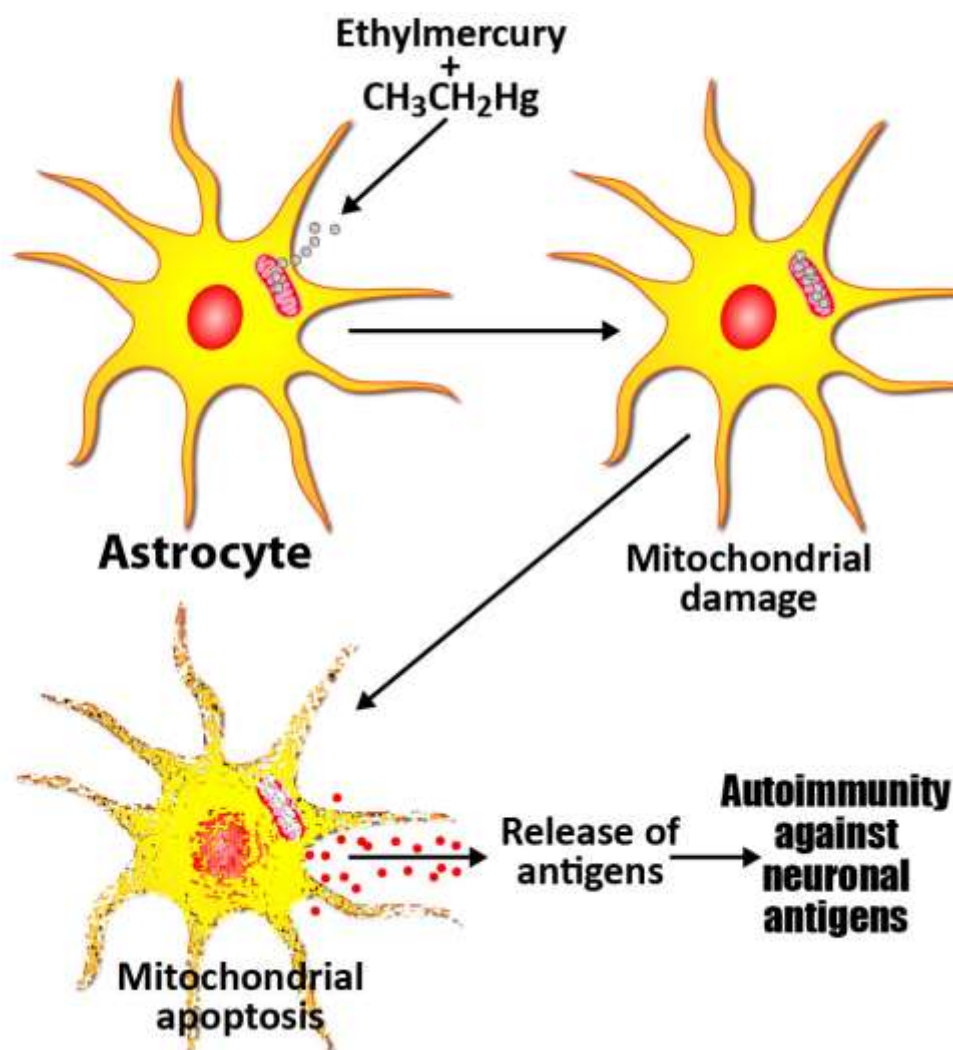
the incidence of ASDs and concluded that thimerosal-containing DTaP vaccines may contribute to an increase in neurodevelopmental disorders. Across studies, mercury has been implicated in sensory motor, immune, neurological and behavioral dysfunctions found in ASD.<sup>64-76</sup> Vaccine manufacturers agreed in 1999 to remove thimerosal from all vaccines except multi-dose influenza or flu vaccines; these still contain 25,000 ppb of mercury.<sup>77,78</sup> Immune reactivity to mercury compounds occurs when EtHg binds to the thiol group in cysteine, forming a complex with various proteins. The neoantigen causes an increase in serum immunoglobulin production and induces immune complex formation, which can lead to autoimmune reactivity (Figure 4). Additional research on the effects of EtHg exposure shows that



**Figure 4. How ethylmercury can combine with a cysteine group to form a mercury-protein complex that can lead to autoimmune reactivity.**

EtHg inhibits mitochondrial respiration and also increases the formation of hydroxyl radicals.<sup>63</sup> Oxidants superoxide and hydrogen peroxide increase cellular levels of aldehyde or ketones and contribute to a five-fold enhancement in mitochondrial DNA damage and increase levels of mitochondrial DNA nicks by 300%.<sup>63</sup> After thimerosal exposure astrocyte mitochondria appear to have a five-fold increase in Caspase-3

activity, which indicates astrocytes are undergoing programmed cell death.<sup>76</sup> The mechanism by which EtHg causes the formation of oxidants has been simplified and illustrated in Figure 5. Mercury exposure can contribute to dysfunctions in the body that manifest in the varied immune, behavioral and neurological characteristics seen in ASDs.



**Figure 5. Mechanism by which ethylmercury can lead to autoimmunity against neuronal antigens.**

Although mercury exposure has been linked to disorders effecting cardiovascular, liver, lung, intestinal tract, renal, immune system, endocrine system, reproductive system and fetotoxicity, it is notorious for its effect on the brain and nervous system. Because mercury can be quickly circulated out of the blood stream and sequestered into different tissues it is important to note here that a direct correlation between blood mercury concentration and the severity of mercury poisoning cannot be used for clinical purposes.<sup>79</sup> However, mercury can damage the blood brain barrier and thus enables the penetration of the brain by mercury and

other toxic metals and substances.<sup>79</sup> Soon after entering the nervous system, mercury quickly becomes tightly bound in the brain, spinal cord, ganglia, autonomic ganglia, and peripheral motor neurons, and therefore cannot be detected in blood or urine levels. It is thought that mercury's ability to cause neuronal problems is facilitated through blockage of the cytochrome P-450 enzymatic process.<sup>79</sup> Mercury is associated with increased tissue oxidative damage, and children with ASD had significantly higher urinary levels of lipid peroxidation, a by-product of tissue oxidative damage, when compared to healthy controls.<sup>80</sup> A

devastating effect of mercury, in the nervous system, is its interference with the production of energy which can impair cellular detoxification processes causing the cell to either die or live in a state of chronic malnutrition.<sup>76,79</sup> Mercury can have an effect on multiple organs and tissues in the human body, which may cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits associated with ASDs.<sup>79,81</sup>

**2.3.2. Aluminum.** This metal is practically ubiquitous in today's world, showing up not only in construction but also in the food and medical industries. A breast-fed infant will ingest around 7 mg of aluminum per day throughout the first six months of life. Another neonate exposure of aluminum comes from vaccinations, as this metal has been used as an adjuvant for about 90 years.<sup>82</sup> Aluminum is present in vaccines such as Hepatitis B (HepB), Hepatitis A (HepA), Diphtheria, Tetanus, and Pertussis (DTaP), *Haemophilus influenzae* Type b (Hib), human papillomavirus (HPV) or Gardasil®<sup>83</sup> and others that are part of the 2016 pediatric vaccination schedule.<sup>84</sup> Unlike dietary sources of aluminum, of which a small percentage (about 25%) is absorbed into systemic circulation, aluminum from vaccines may be absorbed with close to 100% efficiency,<sup>85</sup> which shows that aluminum bound to antigens has different toxicokinetic properties from ionic aluminum found in the environment. Based on vaccine product insert information and the CDC recommended vaccination schedule, at 12 and 18 months of age, babies receive around 1500 and 6000 micrograms of aluminum respectively. Being that the human body, immune system, vital organs and tissues are still developing during this fragile age, does it make sense to knowingly inject aluminum into our children? An increasing number of studies on human and

other animals have demonstrated the toxic effect of aluminum on the GI tract and the musculoskeletal and nervous systems, with the end result being autoimmunity or neuroautoimmunity.<sup>83-100</sup>

Although autism is primarily known as a disorder of the brain, as many as 9 out of 10 suffer from GI problems as well; these include gluten immune reactivity, irritable bowels and increased intestinal permeability. Aluminum exposure has been implicated in both GI and neurological disorders. Recently the effects of aluminum on gut inflammation and the pathophysiology of inflammatory bowel disease was investigated.<sup>85,101</sup> Researchers recorded the effects of environmental doses of aluminum (1.5 mg/kg) in murine models of colitis, where aluminum induced colitis. The authors concluded that aluminum might be an environmental risk factor for irritable bowel disease.<sup>101</sup> When there is dysfunction in the gut, it puts the nervous system at risk. The impact of aluminum on the nervous system has been demonstrated.<sup>88,89,96</sup> Experimental research has indicated that aluminum adjuvants have the capacity to induced long-term neurological inflammation.<sup>87-90</sup> Studies on dialysis-induced dementia<sup>88,89</sup> showed that afflicted patients exhibited abnormally high levels of plasma and brain aluminum, but treatment with chelating agents or removal of aluminum from the dialysis fluid resulted in significant improvement of their symptoms, which included speech disturbance, loss of memory and psychomotor control, behavioral changes, epileptic seizures and coma. Neurotoxicity is induced by aluminum first by its entry into the cytoplasm where it causes hyperphosphorylated tau, neurofibrillary aggregates and tangle formation.<sup>102</sup> Then aluminum enters the nucleus where it induces the generation of free radicals that oxidize and cross-link nucleic acids and



proteins,<sup>103-105</sup> which results in neuronal damage. Despite this knowledge, aluminum continues to be used in food products, food packaging and vaccines, where it may contribute to the autoimmune epidemic we currently face.<sup>93,106-113</sup>

### 2.3.3. Formaldehyde and Glutaraldehyde.

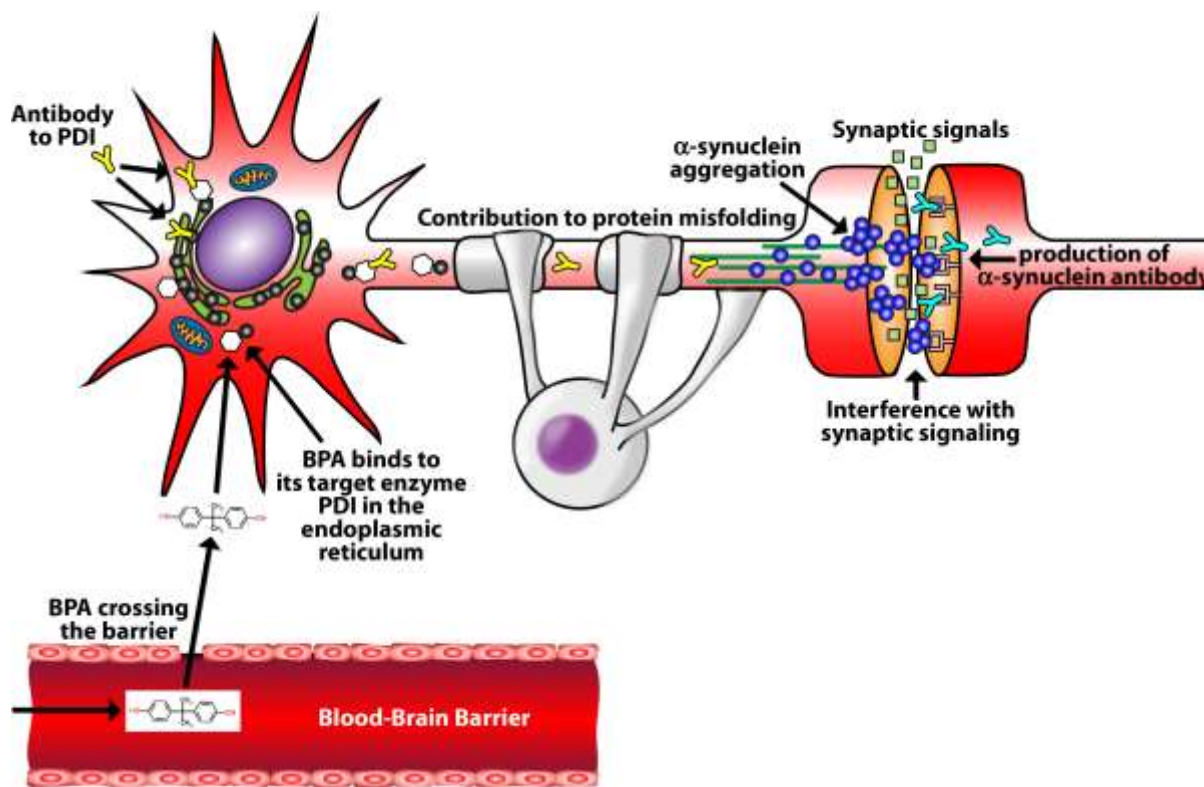
Formaldehyde is a highly reactive chemical that is known to be a carcinogen. Glutaraldehyde is commonly used as a disinfectant and wart cure, although it has other applications. Daily exposures to formaldehyde and glutaraldehyde occur via furniture and paper products, cosmetics and disinfectants.<sup>114,115</sup> The possible routes of exposure to formaldehyde are ingestion, inhalation, dermal absorption and, rarely, blood exchange as in dialysis.<sup>116</sup> Because formaldehyde is so soluble, it is quickly absorbed in the respiratory and the gastrointestinal tracts.<sup>117</sup> Inhalation exposure to formaldehyde has been identified as a potential cause of asthma.<sup>117</sup> Inhalation of formaldehyde leads to the formation of DNA-protein cross-links, DNA single-strand breaks, chromosomal aberrations, sister chromatid exchange and gene mutation in human cells in vitro.<sup>117</sup> This mechanism may be the cause of the carcinogenic, mutagenic and sensitizing action of formaldehyde. In dental practice it is common to have concomitant exposures to formaldehyde and glutaraldehyde.<sup>117</sup> Chronic inhalation of glutaraldehyde caused considerable non-neoplastic lesions in the noses of study rats and mice.<sup>118,119</sup> In our own studies, we found elevated antibody levels to formaldehyde and glutaraldehyde in patients living in a mobile home as well as in healthy subjects, indicating that these chemicals form neo-antigens with human proteins, including HSA.<sup>61,121</sup> Based on earlier studies<sup>120-122</sup> on chemical reactivity coupled with our own, it is possible to conclude that formaldehyde and

glutaraldehyde in their various forms exhibit the ability to react and cross-link proteins and nucleic acids, leading to a broad range of conjugates.<sup>123,124</sup> Strong binding of formaldehyde and glutaraldehyde to human tissue antigen can result in antibody production against formaldehyde and glutaraldehyde as well as human tissue antigens.<sup>61,125</sup> If the chemical exposure continues, autoimmunity can ensue.

2.3.4. *Bisphenol A*. Bisphenol A (BPA) or its substitutes, such as bisphenol S (BPS) and bisphenol F (BPF),<sup>126</sup> are used in a multitude of different plastic products, such as plastic bottles, the lining of cans, and as a coating on some papers,<sup>127-130</sup> and are the most common chemicals to which humans are exposed. This is because storing acidic and even non-acid products in plastic containers leaches BPA into the stored food or drink. Consider the grocery aisle lined with salad dressings, vinegar, soft drinks, oils, alcohol and condiments all packaged in plastic containers. Add to drinking from plastic cups, sports bottles or plastic lids covering hot cups of coffee or tea, and there is no question as to why more than 90% of the US population has detectable levels of BPA in their urine.<sup>131-133</sup> This widespread use is despite the fact that BPA and its substitutes are endocrine disruptors<sup>126</sup> and have the potential to impact fetal, child and adult health. BPA has been shown to bind to hormone receptors such as estrogen and impact signaling as either an agonist or an antagonist, thereby disrupting normal cell functionality<sup>126,127,134,135</sup>. Exposure to BPA even at low doses during gestation has been shown to have long-lasting transgenerational effects on brain mRNA expression and social behaviors.<sup>136</sup> Gestational BPA exposure is associated with hyperactivity and aggression in 2-year-old girls and with anxiety and depression in 3-year-old girls.<sup>137,138</sup> In another study, maternal

urinary BPA concentrations during pregnancy were associated with increased aggressive behavior and emotional reactivity in boys between 3 and 5 years of age.<sup>139</sup> Although the mechanisms of pathophysiology have not yet been elucidated, numerous studies suggest that BPA can alter brain development,<sup>140</sup> and thus may affect the onset and progression of neurological disorders seen in ASDs. This could be due to a specific enzyme in the endoplasmic reticulum of neurons called protein disulfide isomerase (PDI). Because PDI serves as a target enzyme for BPA, it has been identified as a BPA-binding protein. If BPA is able to penetrate the

blood-brain barrier and makes its way into the brain, it can seek out and bind to PDI in the endoplasmic reticulum of neurons. This can result in the production of antibodies against both the BPA and PDI. The antibodies may inhibit PDI activity, resulting in the protein misfolding of  $\alpha$ -synuclein and other signaling molecules. This can cause the formation of  $\alpha$ -synuclein aggregations, which, along with the  $\alpha$ -synuclein antibodies, can interfere with synaptic signaling, resulting in neuroautoimmune and neurodegenerative disorders.



**Figure 6. BPA crosses the blood-brain barrier (BBB) and then binds to its target enzyme (PDI) located in the endoplasmic reticulum, resulting in interference with synaptic signaling and possibly in neuroautoimmune and neurodegenerative disorders.**

**2.3.5. Artificial Food Coloring.** Artificial food coloring, such as Patent Blue, Brilliant Blue, tartrazine, Allura Red, erythrosine,

xanthene and others are made from petroleum and, though known to cause DNA damage, adverse effects on the liver and

kidneys, and have carcinogenic properties, have not been restricted but have actually seen increasing use in a growing number of food and pharmaceutical products for the last 50 years.<sup>141-144</sup> Because artificial food colorings are ionic, they interact profusely with other proteins forming covalent bonds.<sup>145</sup> Unfortunately, covalent binding of food colorings to human proteins, including human serum albumin and hemoglobin, is a major mechanism for the induction of immune reactivity associated with various artificial colorants.<sup>145-149</sup>

Additionally, the covalent binding of food coloring to different food amino acid sequences prevents digestive enzymes from breaking down the food product.<sup>145,150</sup>

Artificial food colorings have significant immunological consequences due to their ability to bind to human tissues and/or prevent effective digestion.<sup>151,152</sup> If the binding of food coloring occurs in the GI tract, the result could be the accumulation of undigested food proteins, gut dysbiosis and enhanced intestinal permeability.<sup>153</sup> Once the gut barrier has been breached, food colorings can enter the bloodstream and bind to a variety of human tissue proteins. This binding mechanism could be responsible for the increased incidence of neurobehavioral disorders found in children and adults.

Between 7-10% of children, worldwide, suffer from some type of hyperactivity.<sup>154</sup>

To assess the correlation between food and behavior, Pelsser *et al.*<sup>155</sup> selected 100 children diagnosed with ADHD, of whom 50 were put on a diet that restricted gluten, dairy, food coloring and other antigenic foods, while the other 50 had no dietary restrictions. After 5 weeks, 78% of the diet group participants scored significantly better in hyperactivity scores than the non-diet group. The improvement in the clinical score of children with ADHD further strengthens the argument that artificial food colorings have a negative impact on behavior. In addition to triggering

hyperactivity, artificial food coloring has been shown to<sup>153</sup>:

- Cause failure in oral tolerance
- Interfere with digestive enzymes
- Enhance intestinal permeability
- Trigger food immune reactivity
- Elicit hypersensitivity to environmental antigens
- Cause allergic rhinitis, asthma and angioedema
- Play a role in atopic dermatitis
- Contribute to liver toxicity and mitochondrial dysfunction

Clearly, artificial food colorings have a far-reaching impact on the human body. Consider the impact they have on the body of a child with ASD, a child who is already over-challenged by so many other environmental insults.

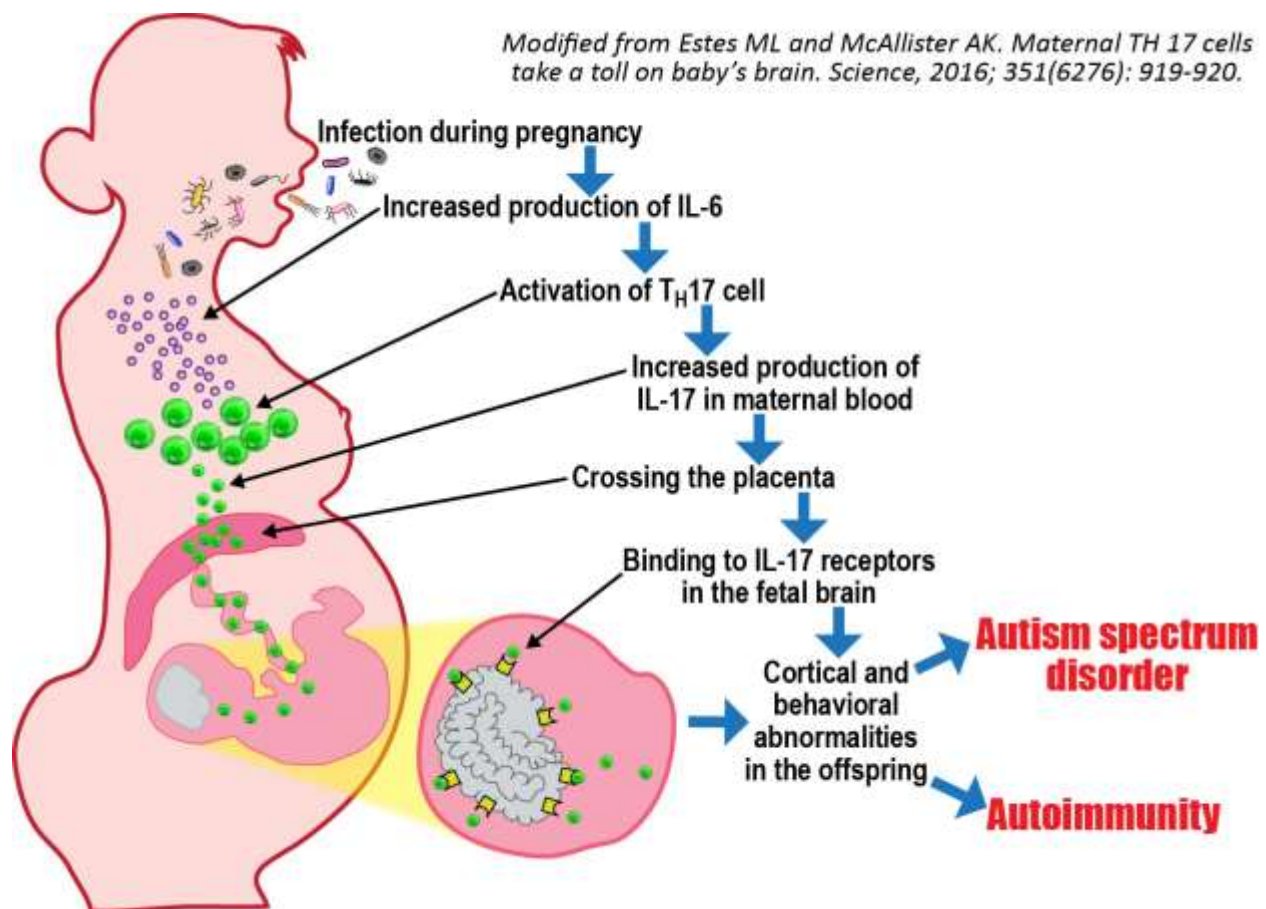
### 3. Pathogens in ASD

Various organisms involved in diseases from ear infections<sup>156</sup> to neuroborreliosis<sup>157</sup> have been implicated in ASDs. Many studies have shown infections to be elevated in ASD children compared to healthy subjects,<sup>158-162</sup> while others conclude there is no significant elevation of antibodies in ASD children.<sup>163-165</sup> In either case, ASD children have been shown to make antibodies to viruses and other pathogenic agents. It is not the quantity of antibodies that is relevant, it is what that pathogen is doing to the body of the ASD child. ASD children, more so than healthy subjects, have increased intestinal permeability, which allows for the increased translocation of gut-bacterial lipopolysaccharides (LPS). LPS, also known as lipoglycans, are large molecules consisting of a lipid fraction and a polysaccharide; they are found in the outer membrane of gram-negative bacteria in the gut and air, act as endotoxins and elicit strong immune responses in humans. Systemic LPS is known to open the blood-brain barrier (BBB). A breach in the BBB is the gateway for circulating pathogens or the antigens made against them to infiltrate the

brain and nervous system. Healthy subjects, with intact barriers, have protection against pathogens entering the nervous system. Researchers are elucidating the pathogenic role LPS plays in disorders of the gut, lung, liver, joints and thyroid as well as nervous, immune and endocrine systems.<sup>166-168</sup>

Libbey and colleagues<sup>159</sup> compiled an extensive review of studies attempting to link viruses to the onset of autism. In their review of 2005, the only virus that appeared to show a connection was congenital rubella virus. In other studies, researchers found that prenatal exposure to rubella caused the offspring to have an altered immune response to the rubella vaccination, and

increased the likelihood of developing ASD.<sup>8,9,159</sup> More recently, however, researchers using a murine model found that pregnant mice injected with polyinosinic:polycytidylic acid (poly(I:C)), an immunostimulant similar in structure to double-stranded RNA, which is found in some viruses, experienced an upregulation of proinflammatory cytokine interleukin-17A (IL-17A).<sup>169</sup> This increase in IL-17A can trigger an immunological cascade (Figure 7) that may affect fetal brain development.<sup>170</sup> The offspring of these mice exhibited behavioral abnormalities similar to those seen in ASD.



**Figure 7. Mechanism by prenatal exposure to bacteria can affect fetal brain development.** Exposure of pregnant mother to bacteria can lead to increased production of IL-6 and activation of IL-17, resulting in an immunological cascade. The IL-17 binds to specific receptors in the fetal brain, which may cause cortical and behavioral abnormalities, ASD and autoimmunity.

#### 4. Breakdown of Immune Tolerance

Immune tolerance is the state of immune non-responsiveness to self-tissue, food antigens, and friendly microbiota. In this state, the immune system does not react to foods or commensal bacterial antigens, but responds to foreign antigens such as chemicals bound to proteins and infectious agents. A functioning immune system protects the body from antigen takeover of the body. Gut-immune homeostasis keeps everything running smoothly, but when imbalance occurs, the mucosal immune system skews, resulting in loss of oral tolerance to environmental antigens. When there is a loss of oral tolerance, the intestinal barrier can break down, and immunogens may enter the submucosa and the blood, igniting immune reactivity to the antigens and switching the immune system from a tolerogenic state to an overactive state. Due to the similarity of food proteins and infectious antigens to human tissues, antibodies produced against the food and infectious antigens may also attack human tissues. The result is autoimmune reactivity due to loss of immune tolerance.<sup>171-175</sup> That is why in individuals with inflammatory and neuroimmune disorders, emphasis should be placed on re-establishing oral tolerance after its breakdown,<sup>48,172</sup> as failure in oral tolerance is the root cause of immune abnormalities detected in autism.

#### 5. Degradation of Barrier Tight Junctions and the Infiltration of Environmental Antigens

The intestinal and blood-brain barriers have two important functions. First, they act as defenses against unwanted antigens, preventing them from infiltrating the body and the brain. Second, they sieve molecules allowing only essential nutrients to pass through the epithelial layer.<sup>176</sup> The barriers are made of epithelial cells connected to each other by a series of tight junction proteins. The most challenged barrier is the intestinal barrier as it is bombarded with

multiple “foreign” and commensal bacterial antigens, foods, and chemicals, on a daily basis.<sup>177</sup> When the intestinal barrier is breached, multiple immunogens can enter the bloodstream, and there, induce inflammatory and antibody responses. The bacterial toxins (LPS), the antibodies, and the inflammatory cytokines may trigger the opening of the BBB. If the bloodstream is home to an array of autoreactive T cells and antibodies, those antibodies and T cells can infiltrate the brain and nervous system, causing damage to neurological tissues. Thus, an intact intestinal barrier is critical to normal physiological function and the prevention of disease.<sup>178,179</sup> Unfortunately, the loss of oral tolerance to foods, gut dysbiosis and body burden of chemicals can inflame the intestine and thus open the tight junctions of the barrier resulting in enhanced intestinal permeability, induction of epithelial cell apoptosis, intestinal barrier breakdown, and entry of not only environmental trigger immunogens, but also intestinal barrier tissues into the bloodstream. Upon entry into circulation, intestinal barrier structure proteins such as, occludin, zonulin, vinculin, talin, actin and actinin can ignite autoantibody responses, which will further damage the intestinal tissue. Furthermore, the BBB is also made of occludin, zonulin, actin and other tissues.<sup>176,180</sup> When antibodies are made against intestinal barrier tissue proteins, these antibodies may mistake BBB tissue proteins as intestinal tissue proteins and begin damaging the BBB.<sup>181</sup>

Indeed, most diseases of the brain are associated with BBB dysfunction. Inflammation induced by environmental triggers is a known cause of BBB disruption. 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 that are normally excluded can penetrate the BBB through tight junction openings and cause neuroautoimmunity with



CNS symptoms.<sup>182,183</sup> Viruses can penetrate the barrier by attaching onto circulating cells of the immune system.<sup>184</sup> Systemic LPS enhances both immune cell and free virus transport across the intact BBB.<sup>185,186</sup> In addition, 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.<sup>187</sup> Therefore, BBB breakdown may precede, accelerate, exacerbate or contribute to chronic disease processes in neurodegenerative disorders including ASD.<sup>188-190</sup>

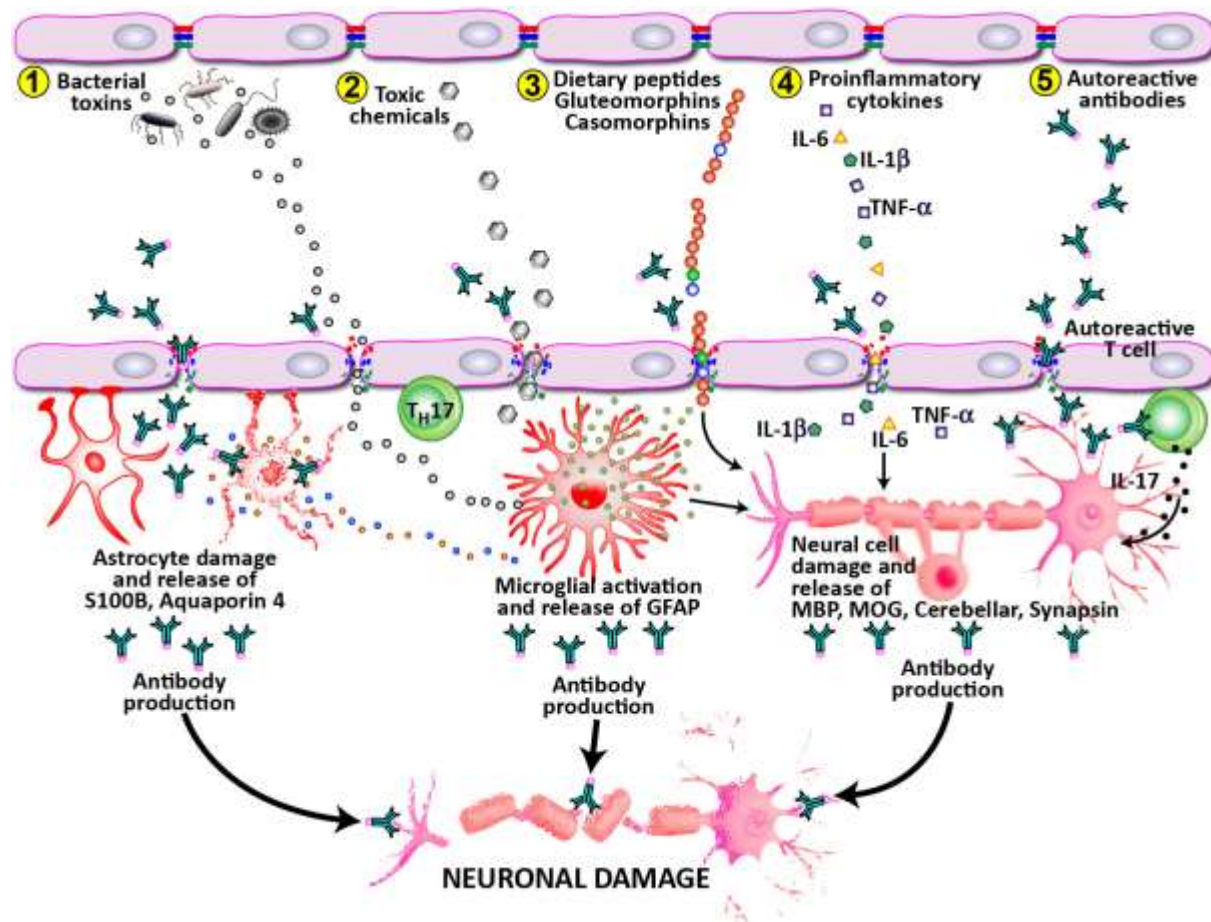
## 6. Autoimmunity and/or

### Neuroautoimmunity

Autoimmunity to brain tissue has been detected in a subgroup of children with ASD and the possible pathogenic role of these antibodies in autism has been discussed<sup>5, 191-195</sup>. Proinflammatory cytokines and astrocytes plus microglial activation may be a possible contributing faction to neuroinflammation and increased frequency of brain-specific autoantibodies in some children with autism.<sup>191-196</sup> Indeed several studies provide evidence that children with autism suffer from neuroinflammation involving different regions of the brain.<sup>5,196-198</sup> Beyond the BBB additional targets of neurodegeneration in ASD may include, myelin basic protein (MBP),<sup>199,200</sup> myelin oligodendrocyte glycoprotein (MOG),<sup>201,202</sup> cerebellar<sup>50,203,204</sup> and synapsin.<sup>205,206</sup> The

production of autoantibodies against these neural antigens leads to neural tissue destruction. When the target of autoantibodies is neurological tissues, the result will be neurodegeneration that can manifest as behavioral problems, movement disorders and communication difficulties seen in ASD.

Neuroinflammation and subsequently detected antibodies against various neuronal antigens in ASD could be the result of a breakdown in the intestinal barrier, and the upregulation of proinflammatory cytokine production. The formation of antibodies against invading dietary proteins/peptides, chemicals, metals, and pathogens-related antigens, followed by the breakdown of the BBB, may open the brain and nervous system to the risk of degeneration. An illustration of this pathogenesis is provided in Figure 8. Along with inflammation as described above, there are two mechanisms by which neuroautoimmunity ensues: 1) Molecular mimicry, or cross-reactivity, occurs when antibodies formed to detect specific environmental immunogens such as gliadin, dairy, cytomegalovirus and *Candida albicans* mistake neuronal tissues for the specific antigen. 2) Environmental factors such as chemicals, metals and lectins/agglutinins may bind to neuronal tissues and ignite an immune response against the tissue to which it is bound. This binding of antibodies to neural antigens may result in the loss of functionality found in autism spectrum disorders.



**Figure 8.** A breach in the blood-brain barrier by 1) bacterial toxins, 2) toxic chemicals, 3) dietary peptides, 4) proinflammatory cytokines, and 5) autoreactive antibodies can lead to the activation of microglia and astrocytes, damage to neuronal cells, and the production of antibodies against S100B, aquaporin, GFAP, MBP, MOG, synapsin, and other neural antigens. Altogether these environmental triggers and immune mediators contribute to neuronal cell damage.

## 7. Conclusion

Although the experts cannot agree on the precise role that environmental factors play in ASD,<sup>2,3</sup> most agree that the combination of genes, environmental triggers and breach of essential body barriers contribute to the multi-faceted characteristics of ASDs. By thoroughly understanding the mechanisms that lead to dysfunctions of the barriers and immune system, healthcare practitioners can better assess and manage their ASD patients and help them on their way to recovery. In this review we have provided multiple aspects of environmental insults that can

promote barrier breakdown, loss of immune tolerance and immune dysfunction. We have shown how body barriers play a vital role in protecting the body and the brain from environmental insults, but when a breach occurs, those sensitive organs can be destroyed by autoimmune mechanisms. Environmental chemicals, metals,<sup>6</sup> and dietary lectins/agglutinins,<sup>207</sup> and bacterial toxins are known to bind to specific proteins or receptors in human tissues. The act of environmental factors binding to human tissue forms a new antigen complex or neo-

antigen that can elicit a strong immune reaction due to its alien characteristics. The act of binding to tissue also breaks down tissue, causing tissue antigens to free-flow through the bloodstream. Here the immune system will identify these tissue antigens as unwanted materials, and thus make antibodies against them. Once the formation of autoantibodies occurs, tissue degeneration may follow. This is autoimmune reactivity. If the exposure to the environmental trigger is not eliminated, the tissue destruction will continue to the point of autoimmune disease. Exposure to environmental triggers, molecular mimicry between environmental immunogens such as dietary proteins, or

bacterial toxins, and tissue proteins, and inflammatory cytokines, individually or synergistically, can damage the BBB, through which cytokines, antigens, and autoreactive T cells can then gain entry, causing damage to the neurons and setting the stage for the neuroautoimmunity detected in ASD. The earlier the environmental triggers are detected and removed, the better will be the clinical conditions of ASD patients.

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