REVIEW ARTICLE

A POSSIBLE CENTRAL MECHANISM IN AUTISM SPECTRUM DISORDERS, PART 1

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The autism spectrum disorders (ASD) are a group of related neurodevelopmental disorders that have been increasing in incidence since the 1980s. Despite a considerable amount of data being collected from cases, a central mechanism has not been offered. A careful review of ASD cases discloses a number of events that adhere to an immunoexcitotoxic mechanism. This mechanism explains the link between excessive vaccination, use of aluminum and ethylmercury as vaccine adjuvants, food allergies, gut dysbiosis, and abnormal formation of the developing brain.

It has now been shown that chronic microglial activation is present in autistic brains from age 5 years to age 44 years. A considerable amount of evidence, both experimental and clinical, indicates that repeated microglial activation can initiate priming of the microglia and that subsequent stimulation can produce an exaggerated microglial response that can be prolonged.

It is also known that one phenotypic form of microglia activation can result in an outpouring of neurotoxic levels of the excitotoxins, glutamate and quinolinic acid. Studies have shown that careful control of brain glutamate levels is essential to brain pathway development and that excesses can result in arrest of neural migration, as well as dendritic and synaptic loss.

It has also been shown that certain cytokines, such as TNF-α, can, via its receptor, interact with glutamate receptors to enhance the neurotoxic reaction. To describe this interaction I have coined the term *immunoexcitotoxicity,* which is described in this article. (*Altern Ther Health Med*. 2008;14(6):46-53.)

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Autism spectrum disorders (ASD) are an increasingly common group of neurodevelopmental disorders without a clearly defined cause. This spectrum of disorders is characterized by a collection of neurobehavioral and neurological dysfunctions often occurring before age 36 months, which include a loss of eye contact, deficiencies in socialization, abnormal theory of mind function, language dysfunction, repetitive behaviors, and some difficulties with executive prefrontal lobe functions.[1,2]

The disorder has a prevalence of males to females of 4:1. A regressive loss of developmental skills occurs in 30%, most often between the ages of 18 months and 24 months. It also has been noted that autistic boys are more likely to experience an early onset of puberty.[3-5] Recent epidemiological evidence indicates a rapid rise in the prevalence of autism, with a 1 in 150 to a 1 in 160 incidence.

Neuropathological studies have shown abnormalities in the architecture of the autistic brain affecting cortical, subcortical, limbic, and cerebellar structures.[6-8] One of the most consistent findings has been hypoplasia of the inferior vermis of the cerebellum with variable but substantial loss of Purkinje cells in the cerebellar cortex.

The bulk of the evidence indicates that immune factors play a major role in these disorders.[9-11] Likewise, abundant evidence implicates mercury neurotoxicity from previously high levels of ethylmercury used as a preservative (thimerosal) in a number of childhood vaccines, as well as other sources of mercury.[12,13]

A host of other observations related to ASD has been aired, including abnormalities in organic acids, opioid-like substances from gliadin and gluten metabolism, intestinal dysbiosis, and trace element imbalances. A strong genetic influence is also known to exist.[14]

Neuroscience has discovered one mechanism that explains most of the findings in ASD: the excitotoxic cascade. New studies have linked a number of seemingly unrelated events to this cascade, such as immune activity, neurohormone abnormalities, and a host of biochemical events.[15,16] Examination of the pieces to this puzzle demonstrate that most fit well into this mechanism.

THE EXCITOTOXIC CASCADE

In 1957, Lucas and Newhouse discovered that monosodium glutamate (MSG)–exposed rats developed degeneration of the inner ganglion layers of the retina.[17] John Olney in 1969 discovered that the food additive MSG could produce delayed neuron death when animals were fed the substance in higher concentrations.[18] He observed not only destruction of the animals' retinal neurons but also destruction of selected nuclei in the hypothalamus and other brain structures. He coined the name *excitotoxin* based on the early observation that the neurons seemed to excite themselves to death in a delayed manner.

The glutamate receptor system consists of 3 ionotropic receptors

(N-methyl-D-aspartate [NMDA], alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid [AMPA], and kainate) and 3 metabotropic receptors types, with a number of cloned subtypes for each receptor.[19] Arrangement and expression of the various subtypes determines function. It is also known that glutamate receptors are not static but rather can be induced.[20]

The various glutamate receptors differ in structure and physiology, with the NMDA receptor operating via a voltage-gated calcium channel and non-NMDA receptors (AMPA/kainate) operating via intracellular calcium signaling. It is the patterns of subunit assembly within the various glutamate receptors that produce the regional differences in the brain's response to glutamate stimulation.[21] Metabotropic receptors act upon G-protein membrane signaling systems. AMPA-type glutamate receptors alter their membrane expression by endocytosis; thus, increased glutamate stimulation increases their synaptic expression.[22]

When the NMDA receptor, which plays a major role in brain development, is activated, it opens a calcium channel, and the rise in intracellular calcium activates a number of enzymes and cell-signaling molecules, including inducible nitric oxide synthase (iNOS), protein kinase C, phospholipases A2, and the eicosanoid cascade. Excessive stimulation can trigger 2 reactions that can lead to neuron death. One is the classic excitotoxic cascade, and the other is glutamate-induced oxidation via glutamate inhibition of the X_C- antiporter.[23] The latter mechanism involves suppression of cystine uptake with a resulting reduction in glutathione generation.

Protection against excitotoxicity is highly energy-dependent. Norvelli et al demonstrated that inhibitors of oxidative phosphorylation allowed glutamate to become excitotoxic at concentrations that normally produced no toxicity.[24] This explains the triggering of excitotoxic neuron destruction during hypoglycemia without glutamate elevation.[25]

Since the original observation by Olney, neuroscientists have discovered that the brain contains abundant glutamate receptors and that excessive stimulation of these receptors can initiate widespread destruction of a number of brain structures.[26-28] Glutamate has been shown to play a critical role in the development of the central nervous system.[29-34] Using glutamate receptor agonist ibotenate, Marret et al demonstrated that NMDA receptor excitotoxicity could arrest neural migration in a developing hamster brain.[35] They found intracortical and molecular layer heteropias, subcortical and intracortical arrest of migration, and ectopias in the molecular layer of the neocortex. At higher doses they found periventricular and band heterotopias. The effects on brain architecture and migration patterns were dose-dependent. The NMDA agonist produced arrest at all levels of the radial migratory corridors (germinative zone, white matter, cortical plate, and molecular layer).

Others have implicated NMDA receptors in migration of granule cells in rat cerebellar slices.[36] This is of particular interest because a number of studies have shown the cerebellum to be one of the most involved areas in autism spectrum disorders.[37,38] Komuro and Rakic have demonstrated that fluctuations in intracellular calcium secondary to voltage-gated NMDA receptor channel activation controls the speed of granule cells migration, with peaks speeding up migration and troughs slowing down migration.[39] It also has been shown that termination of granule cell migration is triggered by a fall in Ca^{2+} levels and that patterns of calcium fluctuation determine migrations to different cortical layers.[40] Because timed peaks and troughs of glutamate brain levels are critical to central nervous system (CNS) development, factors altering these levels can have devastating effects on brain development and maturation.[41]

Prolonged behavioral abnormalities also have been demonstrated with glutamate exposure during development of the brain.[42-44] Of special interest is the finding by Dubovicky et al that raising glutamate brain levels in the early postnatal period in rats produced defects in adjusting to a new environment 21 and 60 days later, something also found in autistic children.[45] In addition to this study, others have found that the toxicity and behavioral effects of neonatal and early postnatal glutamate exposure were significantly more evident in males, with little effect found in the females.[46] Also of interest, the affected males showed little social interest in littermates, had defects in novelty and perceptual mechanisms and an inability to focus attention again, things common to autistic children. These behavioral changes were prolonged, lasting well into adulthood.

Besides the effect on neural migration and maturation, excess glutamate can trigger destructive reactions that can result in a loss of dendrites, synaptic connection, and even neurons. Vargas et al described degeneration in cortical, subcortical, and cerebella in a series of 11 autopsied autistic patients aged 4 years to 45 years.[47] Most consistent in reported neuropathological studies of autism has been the finding of extensive loss of Purkinje cells in the cerebella of autistic patients.[48,49] Several studies have shown that cerebellar deficits, in conjunction with fronto-limbic connections, can explain a number of the behavioral and learning problems seen in autism.[50-52]

With glutamate being the most abundant neurotransmitter in the brain, a complex system exists to protect neurons from excitotoxic destruction. This consists of the glutamate transport proteins excitatory amino acid transporter (EAAT) 1-5, glutamate dehydrogenase, and glutamate decarboxylase. Also involved is the glutamate/cystine X_c- antiporter.[53] The latter is involved in glutamate exchange, with the intracellular movement of cystine used in glutathione synthesis. Excess extracellular glutamate inhibits cystine transfer, resulting in a decrease in intraneuronal glutathione levels.

A loss of glutathione is of special importance because oxidative stress plays a major role in the neurotoxic effect of excitotoxicity. Excessive activation of glutamate receptors can initiate the generation of a number of free radicals, including hydroxyl, peroxyl, and peroxynitrite.[54] Peroxynitrite is especially damaging to the mitochondria, resulting in enhanced reactive oxygen species (ROS) formation.[55,56] In addition, a number of lipid peroxidation products are produced during excitotoxicity, the most harmful being 4-hydroxynonenal.[57,58] Oxidative damage has been shown to occur in the developing brain and is exacerbated by glutamate excitotoxicity.[59]

MICROGLIA, NEUROGENESIS, AND NEUROTOXCITY IN AUTISM SPECTRUM DISORDERS

Microglia represent the resident immune cell of the CNS.[60] Under normal conditions, microglia exist throughout the brain in a resting state referred to as ramified microglia. Any insult to the brain can trigger rapid activation of ramified microglia, resulting in an ameboid morphology. Ameboid microglia can travel via the extracellular space, acting as scavengers of injured and dying cells. During embryogenesis, activated microglia remove pruned neurons and dendritic processes, thus playing a vital role in brain development.

In addition, microglia are antigen presenting cells (APCs) and thus possess a wide assortment of surface antigens and receptors including ß2-integrins, leucocyte common antigen (LTA), immunoglobulin Fcγ receptors, major histocompatibility complex class I (MHC-I) and MHC-II glycoproteins.[61]

Human microglia while in a ramified state constitutively express transcripts for messenger ribonucleic acid (mRNA) of interleukin (IL)–1ß, IL-6, IL-8, IL-10, IL-12, IL-15, and tumor necrosis factor (TNF)–α, as well as macrophage inflammatory protein-1 (MIP-1), MIP-1ß, and monocyte chemotactic protein (MCP)–1. When activated, microglia express inflammatory cytokines (IL-1ß, IL-6, IL-8, and TNF-α), immunomodulatory cytokines (IL-5, IL-12, and IL-15), and antiinflammatory cytokines (IL-10 and IL-13).

In addition, human microglia also contain receptors for each of these cytokines, which can be activated to increase the release of additional inflammatory cytokines. While we still do not understand fully the mechanism of microglial activation, the mitogen-activated protein (MAP) kinase family of enzymes plays an important role.[62] There is evidence that microglia can respond differently to environmental stimuli determined, in part, by which MAP kinase is activated. For example, the extracellular signal-regulated kinase (ERK) is most responsive to growth factors and phorbol esters, while c-Jun N-terminal kinase/stress activated protein kinase (JNK and p38MAP kinase) is activated by stress signals, such as lipopolysaccharide (LPS) stimulation.[63]

In addition to immune cytokines, chemokines, and surface antigens, microglia also secrete a number of neurotoxic molecules, including reactive oxygen species and nitrogen species (ROS/RNS), lipid peroxidation products (LPO), nitric oxide, and 2 excitotoxins—glutamate and quinolinic acid.[64,65] Likewise, a number of these products can also drive microglial activation and further secretion of neurotoxic molecules. This includes glutamate, quinolinic acid, interferons, inflammatory cytokines, chemokines, and ROS/RNS and LPO products.[66-70]

Using purified cultures of second-trimester human fetal microglia, Lee et al demonstrated LPS-induced mRNA for IL-1ß, IL-6, and TNF-α.[71] IL-1ß was a powerful stimulus for microglial activation, which confirms previous studies showing IL-1ß to be a major regulator of microglial cytokine activation and cytokine secretion.[72] This emphasizes the connection between systemic IL-1ß activation by vaccinations and systemic illness and activation of brain microglia.

In sheep, which reach term at 150 days, TNF-α appears first on embryonic day 30 (E30) and IL-1ß at E35-45 in the cortical plate.[73] This and the previous study not only demonstrate that microglial function begins early in embryonic life but that the cytokines play a critical role in neurodevelopment. The highest levels of TNF-α and IL-1ß were present in the subplate zone at the time of most intense synaptogenesis. Further evidence of the importance of cytokines in neurodevelopment arises from the observation of considerable fluctuations in cytokine levels during pregnancy.[74]

Not only do microglia clean up the debris during pruning, they also secrete a number of growth factors that may guide neuronal migration, enhance survival, and promote dendritic arborization.[75] Microglia are known to secrete a number of growth factors, including nerve growth factor (NGF), neurotrophin 3 (NT3), brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), hepatocyte growth factor, and plasminogen. Most are brain region–specific.[76]

It has been proposed that there are 3 basic states of microglia: a neurotrophic state or resting state; a bifunctional state, during which both cytotoxic and neurotrophic molecules are released; and a cytotoxic state during which only neurotoxic molecules are released.[77] Normally, the latter is tightly regulated. There is evidence that microglia play a significant role in neural migration and dendrite development.[78,79]

There also is evidence that excessive microglial activation can disrupt neurogenesis and neurodevelopment.[80-83] This disruption appears to involve inflammatory cytokines as well as glutamate excess.[84,85] Elevated levels of inflammatory cytokines and glutamate have been demonstrated in a number of studies of autistic children and adults.[9-11] In most of the amino acid studies, serum or blood glutamate levels were measured.[86-88] Hamberger et al found significantly elevated cerebrospinal fluid (CSF) glutamate levels in 4 patients having Rett syndrome, which is known to have autism-like neurological symptoms.[89]

One of the hallmarks of the infantile form of autism is an overgrowth of brain with asymmetrical enlargement of the amygdala and cellular abnormalities in the brain stem, cerebellum, hippocampus, frontal lobes, and parietal lobes.[38,90] Neuropathologically, classic autistic patients have a reduced number of Purkinje cells in their cerebellum[91] and abnormally dense packing of neurons in the amygdala and hippocampus.[92,93]

Despite these findings in classic autism, the increasing number of new cases appearing since the early 1980s includes a large number of patients who do not show these dramatic changes in brain gross anatomy using scanning techniques. Yet Courchesne has shown that scanning techniques may not be able to detect less obvious changes in the cerebellum.[94] The difference in severity appears to be the stage at which the insult arises. Postnatal injury is more likely to produce one of the lesser autism spectrum disorders rather than the more neurodevelopmentally severe classic autism.

In humans, a considerable amount of postnatal brain development occurs, with the greatest period of synaptogenesis and pathway development occurring during the first 2 years after birth. Elevated glutamate and cytokine levels secondary to microglial activation would be expected to affect postnatal brain development as well, as evidenced by effects on adult neurogenesis.[95-97]

Considerable interaction exists between microglia, astrocytes,

and neurons. Resting microglia are known to secrete low levels of several cytokines and growth factors that play a role in neuronal maturation, neuroprotection, dendritic growth, and stabilization.[98] When activated acutely, microglia normally secrete both neurotoxic factors and neuroprotective factors.[99] Growing evidence indicates that chronic activation of microglia and astrocytes produces a state of predominant neurotoxicity.[100-102] Activation of microglia can occur from a number of CNS insults and by the presence of cytokines themselves in an autocrine manner.

Of particular concern in the case of autism is the interaction of excitatory amino acid neurotransmitters and cytokines. A number of studies indicate that there is a co-stimulatory interaction between the two.[103-105] One method by which cytokines and excitotoxins interact was shown by Floden et al, in which they demonstrated that TNF-α and glutamate synergistically stimulated neuronal iNOS expression with subsequent peroxynitrite production, which led to neuronal death.[106] Stimulation with either TNF-α or glutamate alone produced no toxicity, but when added together, they produced robust neurotoxicity. Blocking either peroxynitrite or iNOS prevented the toxicity, indicating that peroxynitrite was the toxic factor. Nitration reactions with cellular components and molecules can impair neuronal function.

The NMDA receptors are co-localized with the TNF-α receptors TNFR1 and TNFR2 on the neuron, allowing cross-talk during stimulation. TNFR1 is predominantly neurotoxic, and TNFR2 is predominantly neuroprotective.[107] Neurons contain primarily TNFR1-type receptors. Regarding the co-localized NMDA receptors, it has been shown that subpopulations of NMDA subunits determine the susceptibility of NMDA-dependent death and thus the eventual neurodevelopmental outcome.[108] Similarly, it has been suggested that regional brain susceptibility to immune/excitotoxic injury is dependent on microglial population densities.[109]

Recently, Takeuchi et al demonstrated that TNF-α increases glutamate release from microglia by up-regulating glutaminase, the enzyme responsible for glutamate generation from glutamine.[110] In addition, they demonstrated that release of glutamate was not via glutamate transporters (EAATs) or the X_c^- heteroexchange system but rather by the connexin 32 (Cx32) hemichannel of the gap junction mechanism.

One of the most comprehensive examinations of immune activation in the autistic brain was conducted recently by Vargas et al.[47] In this study, they performed immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbant assays on the brains of 11 autistic patients dying from non-infectious causes. Their ages ranged from age 3 to 45 years. In this study, they examined brain tissue and CSF for both inflammatory and antiinflammatory cytokines, chemokines, and growth factors. Control patients were chosen in the same age ranges and with relatively similar causes of death.

They found the greatest immune-related damage in the cerebellum with extensive loss of Purkinje cells and granule cells in 9 of 10 cerebella examined. No significant histopathological changes were seen in the age-matched controls. Widespread microglial activation was seen throughout the brain with combined microglial/astrocyte activation most prominently seen in the cerebella, anterior cingulate gyrus, and less so, the medial frontal gyrus.

No correlation was found between the degree of changes and age or clinical developmental regression. A mixed pattern of proinflammatory and antiinflammatory cytokines, chemokines, and growth factors were seen, with a predominance of proinflammatory activity. Similar findings were seen in the CSF. The main source of the cytokines was the astrocytes.

The most consistently elevated proinflammatory factor was macrophage chemoattractant protein-1 (MCP-1), which plays a major role in innate immune reactions and is a vital mediator for monocytes, macrophage and T-cell activation, and trafficking into injured zones.[111] Also significantly elevated in both brain and CSF was IL-6, which not only plays a vital role in neurodevelopment, but, when chronically elevated, especially in the presence of excitotoxins, can disrupt brain development and function.[112,113]

Vargas et al noted that none of the autistic brains demonstrated leptomeningeal, parenchymal, or perivascular inflammatory infiltration, suggesting that the systemic immune system was not playing a significant role in the pathological changes, but they also noted that they were seeing the chronic phase and not the acute onset. It is known that with priming of brain microglia, repeated episodes of systemic immune activation can trigger a chronic, exaggerated brain immune response.[114]

There are several explanations for this in relation to systemic immune activation as a triggering mechanism. It may be that mercury and aluminum from vaccines as well as other sources, by accumulating in the brain, are acting as the innate trigger. It has been shown that both of these metals can trigger microglial activation and neurodegenerative effects.[115,116] By accumulating in the brain, they may act as chronic immune stimulants.[117,118] Studies have shown that mercury preferentially accumulates in astrocytes.[119] It is also suspected that infiltrating bone marrow myeloid progenitor cells are converted into microglia during inflammatory brain conditions, which would be even more likely with chronic immune activation, thus explaining the lack of continued systemic immune activation.[120]

It is obvious that activated microglia, with subsequent activation of astrocytes, can result in an environment that is hostile to developing neurons and the development of dendritic connections and synapses. The timing of the injury during the developmental profile of the various neural systems allows for a complex array of final neurological events and neurological syndromes. The competency of the central nervous system's protective mechanisms, especially the antioxidant systems, DNA repair enzymes, and other cellular mechanisms of protection, determines, to a large extent, the final outcome. The competency of the immune system is also a major determinative factor.

Systemic Immune Stimulation and Microglial Activation

A number of studies have reported worsening of neurological disorders with systemic infection, including Alzheimer's disease, multiple sclerosis, frailty in the elderly, and amyotrophic lateral sclerosis (ALS).[121-124] Likewise, experimental studies have shown

microglial activation following systemic immune stimulation. For example, Vereker et al demonstrated degenerative changes in the hippocampus and entorhinal neurons following intraperitoneal injection of LPS in rats.[125]

One study showed that repeated intraperitoneal injection of LPS during a presymptomatic stage shortened the lives of transgenic ALS mice.[126] In addition, peripheral stimulation of immunity with LPS can increase brain cytokine secretion and exaggerate sickness behavior as well as neurodegeneration.[127,128]

Churchill et al demonstrated that systemic IL-1ß could increase brain IL-1ß mRNA and that the effect was blocked by vagotomy.[129] Systemic IL-1ß also increased TNF-α and IL-6 mRNA induction in the nucleus tractus solitarius, hypothalamus, hippocampus, and somatosensory cortex, most of which are affected in the autistic brain. When TNF-α was increased systemically, brain mRNA for TNF-α and IL-1ß were increased in the hypothalamus, hippocampus, and somatosensory cortex, but IL-10, a powerful antiinflammatory cytokine, did not increase. Of particular interest was that IL-1ß increased IL-6, IL-1ß, and TNF-α in the amygdala and decreased the growth factor BDNF in the same nuclei. Amygdalar involvement is thought to play a significant role in the social deficiencies of autistic children.[130]

It is also known that IL-1ß activates neurons in the central nucleus of the amygdala[131] and that the same nucleus plays a major role in hypothalamic-pituitary adrenal (HPA) axis response to systemic immune stimulation.[132] Interestingly, some autistic children have been shown to have low corticoid secretion,[133] and others have hypersecretion of cortisol, thought to be related to stress.[134] This could indicate abnormalities in the glutamatergic-controlled HPA triggered by inflammation and excitotoxicity.[135] Whereas normally cortisol secretion via the HPA controls the immune response, preventing excessive immune-mediated damage, low levels would aggravate chronic microglial activation.

Systemic activation of brain microglia can take place through the blood-brain barrier (BBB)–vascular interface, choroid plexus, and by way of vagal afferents. Likewise, any disruption of the BBB will increase the likelihood of systemic interaction with brain microglia. During early development, even postnatal development, the BBB is considered to be incompetent. Oxidative stress also is known to activate microglia, and systemic LPS has been shown to induce oxidative stress in the brain.[136]

Another important process is priming of microglia. Preexisting microglial activation has been shown to magnify neurodegenerative disease, with subsequent systemic immune stimulation.[114,137] Using ME7 prion protein stimulus, Cunningham et al found that priming of microglia in the hippocampus in mice followed by systemic intraperitoneal challenge with LPS produced a 3-fold higher increase in brain IL-1ß than when microglia were not primed.[138] IL-1ß, being the central controlling cytokine for microglial activation, at these high levels would explain the chronic activation seen in the Vargas study. TNF-α levels increased to 1.7 times greater than unprimed state and IL-6, 3 times greater. Interestingly, no difference was found in TGF-1ß secretion, a major immune modulator, with it being elevated equally in the primed and unprimed state. This, as in the study by Vargas et al, demonstrates a predominance of an inflammatory cytokine profile.

In the autistic patient, this would be similar to one of several situations. It has been observed that autistic children have early and repeated systemic infections, usually middle ear infections. These infections would serve to prime the microglia. A subsequent vaccination or vaccinations would be expected to produce an exaggerated microglial reaction based on the priming effect, and each inoculation would prime the microglia for the next inoculation.

Another situation would be inoculating children with live vaccines, such as the MMR (measles, mumps, and rubella) vaccine. It has been shown that the measles virus is retained in the brain over a lifetime following early exposure.[139] Once the virus was established within the brain, subsequent MMR vaccines could trigger an exaggerated immune response in the primed microglia. Even the presence of nonviable viral components could act as activators of microglia.

In addition, retention of vaccine adjuvants in the brain, such as mercury and aluminum, also would act to prime brain microglia. Additional inoculations, especially if spaced close together, would be subject to this priming effect. It has been shown that vaccine adjuvants can cause prolonged activation of the systemic immune system.[140]

Taken together, this evidence clearly indicates that systemic immune stimulation can activate the brain's microglia and that priming of the microglia can cause a magnification of the brain's immune response. It is also clear that repeated systemic immune activation further enhances the destructive nature of CNS immune activation, especially if prolonged. Since the developing child's immune system, including the microglia, are active during early development, excessive and repeated activation can significantly interfere with brain development and function, as discussed earlier.

THE GASTROINTESTINAL SOURCE OF CHRONIC IMMUNE STIMULATION

That chronic inflammation plays a significant role in autism is beyond dispute; the source or sources of chronic stimulation are less clearly defined. As we have seen, recurrent vaccination with powerful immune adjuvants can produce prolonged and intense activation of brain microglial immunity and neurodegeneration, and "primed" microglia exhibit exaggerated immune reactions. It also has been suggested that another source of immune activation can be from food allergies[141,142] or chronic intestinal infections with *Candida* or other dysbiotic organisms.[143]

Wakefield et al suggested a relationship between severe intestinal inflammation secondary to MMR vaccines and autism.[144] They proposed that intestinal inflammation produced malabsorption. A more recent study by Ashwood and Wakefield found that peripheral blood lymphocytes as well as mucosal CD3+TNF-α and CD3+IFN-γ cytokine responses were significantly increased in children with autism spectrum disorder as compared to noninflamed control children.[145] The critical difference between children with Crohn's disease and those with autism spectrum disorder was that in the latter, peripheral and mucosal IL-10 responses were markedly

lower. This indicated not only a gastrointestinal autoimmune reaction in autistic children, but a suppression of the cytokine known to regulate immune termination, IL-10.

Several studies have shown a cross-reaction between food-derived proteins and neuron specific antigens. Vojdani et al examined 9 different neuron-specific antigens and 3 cross-reactive peptides, which included milk proteins, and found that autistic children had the highest IgG, IgM, IgA antibody reaction to all 9 neuron antigens as well as a cross-reaction to all 3 peptides.[146] In a follow-up study in which they assessed the reactivity of sera from 50 autistic patients as compared to 50 healthy controls, Vojdani et al demonstrated that a significant number of the autistic children expressed antibodies against gliadin and cerebellar Purkinje cells simultaneously.[147]

In a number of studies, reactions to commonly found colon bacterial organisms are seen to occur.[146,148] *Candida* infections are often seen in children with autism spectrum disorders and may also act as a source of strong, chronic immunologic reactivity, especially if they penetrate the gut wall.[149,150] The presence of beta-1,6 glucan in the cell wall of the organism appears to be the most powerful immune component.

In a study of 96 autistic children compared to 449 healthy children, Black et al found no greater incidence of gastrointestinal disease in the autistic children.[151] Their study included only obvious gastrointestinal disease and symptomatology and recognized that they might miss more subtle symptoms of gastrointestinal disease.

Another disorder that has shown a strong connection between immunological reactivity to food-based peptides and neurological dysfunction is celiac disease.[152] Patients with this disorder are sensitive to gluten-containing diets (wheat, barley, and rye). Approximately 6% will present with neurological complications, most frequently cerebellar ataxia. Bürk et al not only found cerebellar ataxia and oculomotor disturbances but also subtle cognitive impairments and difficulty with the Wisconsin Card Sorting Test, which is indicative of executive prefrontal deficits.[153] The MRI scan demonstrated atrophy of the cerebellum.

Of interest is that several researchers have noted a lack of gastrointestinal symptoms in a number of these patients. For example, Hadjivassiliou et al found gastrointestinal symptoms in only 13% of patients with MRI evidence of cerebellar atrophy and clinical findings of sporadic cerebellar ataxia.[154] The MRI changes were not limited to the cerebellum, as they also found white matter hyperdensities.

Sporadic ataxia is the most common form of ataxia, and this study found that gluten sensitivity accounted for 41% of cases, making it the most common cause of ataxia. Hu et al examined 13 patients with celiac disease with neurologic involvement and found cognitive impairment in all patients.[155] Slow, progressive neurological onset was characteristic, with development of acalculia, confusion, personality change, and amnesia.

Two of the patients underwent brain biopsy, and 2 came to autopsy. All demonstrated gliosis, indicating microglial activation. One patient demonstrated the findings of frontotemporal lobar degeneration with histological involvement of the frontal and temporal cortices and hippocampal dentate granular cell layer. Not all patients improved with gluten-free diets, due to either poor compliance or progressive neurodegeneration, as is seen with such immune-related neurological diseases as multiple sclerosis treated with immune-suppressing drugs. It is suspected that progression is secondary to chronic excitotoxic neurodegeneration.[156]

Hadjivassiliou et al tested the sera from cases of gluten ataxia, including patients with newly-diagnosed celiac disease without neurological involvement, patients with other cerebellar diseases, and healthy controls using immunostaining with IgG antigliadin antibody on human cerebellar and rat CNS tissue.[157] They found that 12 of 13 of the cases of gluten ataxia stained the Purkinje cells intensely. Some of the cases of celiac disease without neurological symptoms stained the Purkinje cells mildly. No staining was seen in controls. This indicates that patients with gluten ataxia have antibodies against Purkinje cells.

Taken together, these studies clearly indicate that allergies to food components and colon microorganisms can activate CNS microglial innate immunity, resulting in a diverse array of neurological disorders and behavioral changes. They also indicate that obvious gastrointestinal disorders do not have to exist. With priming of the microglia, as would occur with recurrent infections or repeated vaccinations early in life, the intensity of the brain's immune response to food-based peptides would be drastically enhanced and act as a continuous source of brain immune stimulation.

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