Chronic Microglial Activation and Excitotoxicity Secondary to Excessive Immune Stimulation: Possible Factors in Gulf War Syndrome and Autism

Russell L. Blaylock, M.D.

ABSTRACT

There is considerable and growing evidence that chronic microglial activation plays a major role in numerous neurological conditions including Alzheimer's dementia, Parkinson's disease, ALS, strokes, and inflammatory brain diseases. The release of toxic elements from activated microglia, such as cytokines and excitotoxins, is known to produce neurodegeneration. Peripheral immune stimulation has been shown to activate CNS microglia, and when excessive can lead to neurodegeneration and cognitive defects commonly associated with both the Gulf War Syndrome (GWS) and autism. This paper summarizes the mechanism linking these two disorders with excessive immune stimulation secondary to overvaccination.

Clinical Features of Gulf War Syndrome and Autism

Following the first Gulf War, tens of thousands of American and British veterans began to suffer from numerous complaints, which have become known as the Gulf War Syndrome (GWS). These included recurrent fevers, cognitive difficulties, neuropsychiatric problems, polyarthralgias, chronic fatigue syndrome, and numerous allergies where none existed before.

In addition, an increase in birth defects has been documented in the offspring of Gulf veterans, including tricuspid valve insufficiency, aortic valve stenosis, renal agenesis or hypoplasia, and hypospadias.[1]

One recent study documented reduced cerebrovascular flow and impaired ability to handle complex cognitive tasks in a substantial number of these veterans.[2] Another study found that cognitive symptoms correlated closely with immune dysfunction in Gulf War veterans, but not with nonveteran chronic fatigue controls.[3] The Gulf War veteran was exposed to as many as 17 inoculations over a very short period.

In many ways, behavioral problems associated with this syndrome resemble astheno-emotional disorder, which is characterized by fatigue with prolonged mental activity, stress intolerance, decreased concentration and short term memory, headache with mental processing, irritability, and sensitivity to sounds or lights.[4] The anatomic substrate of this syndrome–ascribed to the hippocampus, limbic connections, temporal lobes, and parts of the frontal lobes–overlaps those most damaged by neuroinflammation and attendant excitotoxicity.

In the instance of autism, the significant and unexplained increase beginning in the 1980s paralleled the introduction of a host of new vaccines,[5] although vaccine authorities do not accept a causal connection. The neurologic dysfunction seen in autism and the autism spectrum disorders, like that of the GWS, involves the limbic connections. Because of the rapid development of the child's brain and the immaturity of various biochemical systems, symptoms are seen in the autistic child that would not be expected in the adult, especially echolalia, inappropriate laughing and giggling, and various language-related symptoms. As with GWS, significant immune dysfunction is seen in cases of autism.

The Effect of Immune Stimulation on the Central Nervous System

There is growing evidence that overstimulation of systemic immunity can produce deleterious effects on nervous system function, including neurodegeneration. While most are aware that autoimmunity can occur when nervous system components are involved in immune reactions–for example, with postvaccinal encephalitis and subacute sclerosing panencephalitis–few are aware of chronic neurodegeneration without autoimmunity. Recent evidence indicates that a different type of reaction can occur that may have relevance not only to autism spectrum disorders and GWS, but also to neurotrauma, ischemia, and various neurodegenerative diseases.

McGeer and co-workers have recently defined an immune process not involving autoimmunity, but rather a nonspecific immune destruction of neurons, neurites, and synaptic connections,[6] referred to as autotoxicity. In this process, either systemic immune factors (cytokines) or local immune factors, such as ß-amyloid or viral components, can activate the brain's immune system via activation of astrocytes and microglia. In both instances brain levels of complement, cytokines, reactive oxygen and nitrogen species, cellular immune components, proteases, adhesion molecules, excitotoxins, arachidonic acid, and other chemokines are released. These toxic compounds cause bystander injury to surrounding normal neural elements.

The Role of the Microglia

The central nervous system's immune system is controlled by the microglia, a diffuse set of normally resting cells that when activated can assume ameboid activity and secrete numerous cytokines, chemokines, eicosanoids, proteases, complement,

and at least two excitotoxins. Under most acute conditions and during neurodevelopment, the immune cytokines can act as neurotrophic substances, protecting and promoting neurite growth. With intense activation and when chronically activated, these cytokines and other secretory components of the microglia can be very destructive.

High concentrations of interleukin-1ß (IL-1ß) injected into the brain can cause local inflammation and degeneration of neurons,[7] and even small concentrations in the presence of ß-amyloid, ischemia, trauma, or excitotoxins, can trigger destructive reactions.[8]

The key role of the microglia is demonstrated by the fact that mixed cultures of neurons and microglia will produce intense destruction of the neurons when lipopolysaccharide is added. This does not occur in the absence of microglia.

Activation of microglia has been shown to be an early event, both in experimental models and in human cases of Parkinson's and Alzheimer's diseases.[9,10] In fact, behavioral changes and clinical effects occur before the appearance of amyloid plaques and neurofibrillary tangles in the case of Alzheimer's disease. That these inflammatory processes can be aggravated by systemic activation of microglia has been recently shown in trials of experimental amyloid-beta (Abeta) vaccines used in Alzheimer's patient volunteers in whom several cases of encephalitis were seen.[11]

Microglial activation as a central destructive process has been demonstrated in a growing list of conditions, including multiple sclerosis (MS), Alzheimer's dementia, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Huntington's disease, supranuclear palsy, macular degeneration, glaucoma, trauma, strokes, viral encephalopathy, human immunodeficiency virus (HIV)-associated dementia, and prion disorders.[12] It is also known that IL-1ß can greatly aggravate experimental allergic encephalitis (EAE) when injected into the spinal cord,[13] and that blocking tumor necrosis factor-alpha (TNF-alpha) and IL-1ß can significantly reduce the destruction of EAE.

Many events can activate the microglial immune cytokines, including introduction of heavy metals, aluminum, oxidized LDL, amyloid, viruses, mycoplasma, bacteria, and glutamate, and that the entry point on the microglial membrane is the mitogen-activated protein kinase (MAPK) and its stress protein enzyme (SAPK).[14]

Once microglia are activated, eicosanoid production is also elevated, primarily through the cyclooxygenase-1 (COX-1) enzyme.[15] At the same time there is increased secretion of arachidonic acid and production of prostaglandin E2, a powerful inflammatory cytokine. Release of these destructive elements in the zone of attack spreads to surrounding areas, causing what is called bystander damage, the extent of which depends on the intensity of the microglial activation and how long it persists. In most instances, activation of microglia terminates rapidly, thereby minimizing bystander damage. With chronic microglial activation, bystander damage can be extensive. This is what appears to occur in autism disorders and GWS.

Elevations of brain IL-1ß have been shown to interfere with long-term potentiation (LTP), a critical process in memory development.[16] IL-2 and IL-6 have also been implicated in altering memory and learning.[17] This is especially so in stressful situations, such as would be seen in a theater of war. Other studies have shown reduced hippocampal neurogenesis with chronic elevations of IL-6 and tumor necrosis factor-alpha (TNF-alpha).[18] This would be of special concern in infants and young children.

Microglial activation is known to occur in all of the viral encephalopathies and even spongioform encephalopathies.[19,20] This is particularly important when considering that the persistence of measles virus in the brain is known to occur in 20 percent of the cases of exposure to the virus. This is much higher than is clinically evident. It is not even necessary that the virus enter the neurons, as with HIV-1 dementia. In this instance a viral protein fragment (gp41) is sufficient to activate brain microglia, leading to dementia. *Herpes simplex* virus type 1 (HSV-1) has also been shown to produce brain degeneration by the same mechanism.

The Role of Excitotoxicity

Damage to neuronal mechanisms is not limited to direct cytokine effects. The excitotoxicity initiated by microglial activation is even more important. In cases of HIV-1 dementia, it is known that brain quinolinic acid, an excitotoxin secreted by activated microglia, can increase more than 300-fold.[21] In addition, glutamate is released in concentrations that are known to destroy neurons and/or synaptic connections.

Because glutamate can also activate microglia and enhance cytokine-induced neurodegeneration,[22] a vicious cycle is created in which immune cytokines can stimulate release of glutamate, and glutamate in turn enhances cytokine production and release. Moreover, cytokines inhibit glutamate transporters, which play a critical role in removal of excess extracellular glutamate. Intimately linked to excitotoxicity is the generation of destructive free radicals, especially the reactive nitrogen species such as peroxynitrite and nitrosoperoxycarbonate.[23] Much of the injury to dendrites, synapses and neurons, by both cytokines and excitotoxicity, is caused by free radicals.

The reactive nitrogen species are responsible for nitration of DNA residues, tyrosine, tryptophan, amines, and metalloproteins, thereby producing widespread disruption of numerous biochemical processes. In particular, peroxynitrite plays a major role in suppressing mitochondrial energy production. This not only interferes with brain function, but also greatly enhances excitotoxicity. In fact, it has been shown that when neuron energy supplies are low, even physiologically normal concentrations of excitatory amino acids can become excitotoxic.[24]

Excitotoxicity is also greatly enhanced by low magnesium levels, which have been reported in most cases of autism and are frequent in cases of stress, as would be seen in a Gulf War veteran. In fact, by removing magnesium from a cerebral tissue culture, excitotoxic sensitivity is increased 100-fold, just as with energy

depletion. In addition, low magnesium levels have been shown to enhance release of inflammatory cytokines.[25]

Injecting excitotoxins into the brain has been shown to increase expression of IL-1ß messenger RNA and its protein (IL-1ß) and that when both excitotoxins and IL-1ß are infused into the striatum of the brain, significant destruction occurs some distance away in the cortex as well as at the site of injection.[26] This finding demonstrates the widespread nature of the damage caused when combining immune stimulation and excitotoxicity, which is the effect of chronic microglial activation.

Another particularly destructive product is 4-hydroxynonenal (4-HNE), an aldehydic lipid peroxidation product found in abundant supply in cases of neurodegeneration. Interestingly, peroxylnitrite and 4-HNE are found in the same neurons. This lipid peroxidation product has been shown to be especially destructive of synaptic connections and mitochondrial enzymes and can significantly inhibit glutamate transport proteins. It is neutralized only by alpha-lipoic acid, glutathione, and certain flavonoids.

Elevations in brain glutamate and aspartate impair memory retention and damage hypothalamic neurons in both infant mice and adults.[27] This can be explained by the fact that the hippocampus and amygdala contain numerous glutamate-type receptors, which are thought to play a central role in learning and memory via LTP. In addition, their limbic connections, frontal and cingulate, play a major role in emotional elaboration. Moreover, microinjections of excitotoxins into the ventral subiculum of the hippocampus increase locomotor activity, strongly resembling that seen in some autistic children and attention deficit hyperactivity disorder (ADHD).[28] Hyperresponsiveness to stressful stimuli can be produced by excitotoxic damage to the ventral hippocampus at a very early age, something that would be expected in cases of autistic hyperimmune responses.

The consequence of damage to synapses, dendrites, and cell bodies would be different in the developing brain, especially during the period of the brain growth spurt from the last trimester of pregnancy to age two years. It has been shown that excitotoxicity cannot only disrupt neural elements and function but can alter brain pathway development, resulting in a "mis-wired" brain.[29]

In the adult, one would expect to see impairment of attention/memory, depression, and social withdrawal with similar injuries to the limbic system.

Neurologic and Behavioral Effects of Cytokines

Some of the best information we have concerning cytokine effects on brain function comes from their use in treating hepatitis and cancer patients. The effects of these substances are divided into acute and chronic. The acute effects resemble influenza and persist for one to three weeks. Chronic effects can occur with all dosages, routes of administration, and schedules. Higher doses are more likely to produce more profound and persistent effects.

A growing number of human studies have shown dramatic alterations in learning, memory, attention, and affective state, as well as perceptual and motor functions following cytokine treatments.[30] ALS patients treated with interferon have demonstrated significant impairment of verbal memory and calculation ability.[31] In another study involving 44 cancer patients receiving IL-2, it was found that 71 percent receiving the high dose developed mild-to-severe behavioral changes, including cognitive dysfunction.[32] All these behavioral symptoms resolved within three days of treatment cessation.

Patients receiving more than 1 million units of interferon–alpha show some constitutional symptoms. Chronic symptoms are seen with all doses, but are more likely at doses greater than 18 to 20 million units.[33] Renault et al. divided the behavioral symptoms into three categories: organic personality syndrome, organic affective syndrome, and delirium.[34]

One set of symptoms seems to be similar to those of autism spectrum patients, including uncontrollable overreaction to minor frustration, marked irritability, and a short temper. Gulf War veterans commonly display organic affective symptoms such as depression, feelings of hopelessness, and uncontrollable crying spells. Clouded consciousness, disorientation, irritability, and mood alterations similar to that seen in both autism patients and Gulf War veterans were also seen. In addition, patients receiving the interferon-alpha developed periods of severe agitation, became abusive, and often withdrew from others–other findings they have in common with the autistic child.

Psychomotor retardation was seen in 47 to 80 percent of patients treated with interferon-alpha, along with social withdrawal.[35] Cognitive changes also occurred, with a shortening of attention span, impaired short-termed memory, and mental fog. Other reports describe patients who exhibit periods of silence, and without warning stare vacantly even in mid-sentence.[36]

Most cognitive changes have been reported to be reversible, but some reports describe persistent cognitive problems lasting up to two years following cessation of therapy. In rare instances, patients will become fully demented.[37] This is understandable when we recall that interferon can activate excitotoxicity and produce severe free-radical injury to synapses and neurons. Interferon-gamma is reported to increase superoxide levels. Slowing of thought processes, confusion, and even Parkinsonian symptoms have been reported in patients using interferon-gamma.[38]

IL-2, used to treat infectious disease and cancer, has been shown to result in mental status changes, agitation, combativeness, hallucinations, difficulty concentrating, and delusions. IL-1 has been associated with ideational delusions, seizures, agitation, and somnolence, while TNF-alpha can cause transient amnesia, hallucinations, and even aphasia.

As we have seen, both IL-1ß and TNF-alpha can block glutamate reuptake, resulting in high levels of toxic extracellular glutamate.[39] Cognitive defects are also common following IL-1ß based on inhibition of LTP, which is essential for memory acquisition.

The Role of Vaccines

As stated above, peripheral immune stimulation readily activates the brain's immune system. In most instances this is short-lived, and neuron damage is minimal. Chronic activation of microglia, however, can lead to substantial disruption of neuronal function and even neurodegeneration.

Two basic processes seem to be responsible for the chronic stimulation of brain immunity: repeated, closely spaced inoculation without allowing brain recovery, and inoculation with live viruses or contaminant organisms that persist in the brain. Gulf War veterans were given some 17 inoculations very close together. Children are often given as many as five to seven inoculations during one visit to the pediatrician's office, several as combined vaccines, such as measles-mumps-rubella (MMR).

Of particular concern is the use of live organisms and contaminant organisms. Garth Nicolson and co-workers have demonstrated polymerase chain reaction (PCR) evidence of mycoplasma species in the blood samples of Gulf War veterans suffering from ALS, the incidence of which was found to be increased by 200 percent in this population.[40] Nicolson et al. found that 83 percent of veterans with ALS had positive tests, whereas positives were rarely seen in controls. It is hypothesized that the vaccines were contaminated primarily with *Mycoplasma fermentens*. Numerous activated microglia are found in the spinal cord of affected veterans. The involvement of live *M. fermentens* could also explain the appearance of similar illnesses in other household members.

Excitotoxins contribute to the damage in central nervous system infections. Cerebrospinal fluid glutamate levels rise in bacterial meningitis, and levels are directly correlated with prognosis. Extracellular glutamate levels are elevated in all cases of viral encephalopathies, including that of the acquired immunodeficiency syndrome (AIDS). Glutamate and aspartate levels in the plasma were also found to be elevated in 11 of 14 autistic children.[41] There is also evidence that viruses can enhance the toxicity of glutamate.[42]

Injection of the immune adjuvant lipopolysaccharide (LPS) closely resembles the vaccination process. In one study, it was shown for the first time that peripherally administered LPS decreased learning in mice.[43] The dose used did not produce observable injury to the neurons, but significantly impaired the animals' completing the Morris water maze and spontaneous alternation Y-maze, which tests spatial learning requiring a functional hippocampus. Associative learning was affected most. Memory retention was spared. LPS injection, by elevating IL-1 levels, has been shown to alter hippocampal norepinephrine and serotonin levels, as well as increasing glutamate levels.[44] Elevated serotonin levels have been described in autism.[45]

Long-term persistent immune activation and low-grade brain inflammation have been described in three children who recovered from *Herpex simplex* encephalitis before age two.[46] The children all demonstrated abundant activated microglia at brain biopsy and continued to deteriorate after viral treatment, indicating continued microgial activation. Viral fragments, without active infection, can produce this phenomenon.

Not all persistent viral infections are associated with obvious inflammatory responses. Using a hamster neurotrophic strain of measles, it was found that a noninflammatory encephalopathy could occur with destruction of the CA1 and CA3 segments of the hippocampus.[47] This could more closely resemble the situation in autistic child and some cases of GWS, since obvious clinical and laboratory signs of inflammation would be absent. Neurodegeneration caused by this neurotrophic measles virus was blocked using the NMDA receptor antagonist, MK-801, indicating an excitotoxic mechanism. (The NMDA receptor is the postsynaptic receptor for L-glutamate that can be activated by the drug N-methyl-D-aspartate.)

The smallpox vaccine is associated with postvaccinal encephalitis at a rate of 1 in 110,000 vaccinations. This includes only obvious cases of encephalitis; more chronic, subtle cases involving ill-defined neurological symptoms remote from the vaccination would be overlooked. Most vaccine follow-up studies do not extend beyond two weeks. It is obvious from the above studies that this follow-up period is far too short. More persistent neurotropic viruses now being discovered appear to be related to chronic neurodegeneration. These include HSV-1, coronavirus, measles virus, and human herpes viruses 6 and 7 (HHV-6 and HHV-7). A postvaccinal encephalopathy has been described in children under age two years following the smallpox vaccine.[48] Most of these occur as chronic conditions.

A Multifactorial Problem

Only part of the population exposed to these pathogenic factors have symptoms, and not everyone is affected in the same way. There is a critical interplay among many factors, including mercury exposure, exposure to other toxins (pesticides, chemical warfare agents, and insect repellants), other infectious agents, nutrition, antioxidant system status, and immune function.

Deficiencies in certain nutrients appear to be particularly common in the autistic child, including magnesium, pyridoxine (vitamin B6), and docosahexaenoic acid (DHA), an essential omega-3 fatty acid. Such deficiencies can enhance excitotoxicity. Vitamin B6 has been shown to lower blood and brain tissue glutamate levels. Magnesium acts at the NMDA receptor to down-regulate calcium entry into the neuron. DHA plays a particularly important role in cellular membrane function, especially mitochondrial membranes, and reduces excitotoxicity. Recent studies have also shown that it plays an important role in synaptic membranes as well.

Immune Dysfunction in GWS, Autism, and Other Behavioral Disorders

There is growing evidence that immunologic dysfunction plays a major role in both autism and GWS. Shifting the immune system from a Th1 profile to a Th2 pro-autoimmune profile has been described in the Gulf War veteran and in autistic children.[49,50] There is also compelling evidence that GWS is related to exposure to the vaccine adjuvant squalene.[51] In the autistic child, many observers have noted an increase in ear infections and upper respiratory infections.

There is growing evidence of autoimmunity in autism and other possibly related conditions, including ADHD. Warren and coworkers have described a common link in autism, ADHD, and dyslexia with the finding of an increased frequency of the C4B null gene (which does not produce the complement protein C4B).[52] Others have reported an increase in antibodies to basic myelin protein and neuronal axonal filaments in the brain.[52] Elevations of TNF-alpha have also been described in autism.[54]

This set of immune deficiencies would lead to direct cytokine activation of microglia by way of autoimmune-induced cytokine stimulation of microglial receptors. The immune deficiency caused by low levels of C4B complement, which is important in eliminating viruses, mycoplasma, and fungi would increase the likelihood of viral and mycoplasmal persistence in the brain. In both instances chronic activation of microglia would occur. Many cases of autism and GWS do not involve autoimmunity, but rather bystander injury.

Conclusions

The evidence suggests that overstimulation of the systemic immune system, as by repeated inoculations spaced close together, can result in chronic activation of brain microglia, the nervous system's immune mechanism.

There is abundant experimental and clinical evidence that elevations in cytokines can result in disruptions of brain function, both by generation of numerous free-radical types, and by release of the excitotoxins glutamate and quinolinic acid from activated microglia. Once activated, a complex interplay of oxygen and nitrogen stress, excitotoxicity, and immune cytokines alters synaptic connections, dendrite maintenance, and neuron function, leading to a myriad of symptoms like those seen in GWS and autism.

Both syndromes manifest an impaired peripheral immune system, a possible consequence of excessive vaccination itself, neurotoxic vaccine additives (aluminum and mercury), and immune-suppressive viruses such as the measles virus. This should serve as a caution to those who would add even more vaccines to a schedule already too crowded, as well as an indication to reassess the current schedule.

Russell Blaylock, M.D., is a retired neurosurgeon, now involved full time in nutritional research and writing. He is President of Advanced Nutritional Research, Jackson, Miss. Address correspondence to 315 Rolling Meadows Rd., Ridgeland, MS 39157.

REFERENCES

1. Araneta MR, Schlangen KM, Edmonds LD, et al. Prevalence of birth defects among infants of Gulf War veterans in Arkansas, Arizona, California, Georgia, Hawaii, and Iowa, 1989-1993. Birth Defects Research Part A. *Clin Mol Teratol* 2003;67:246-260.
2. Bugegin L, Mitzel HC, Miller CS, Gelineau JF, Tolstykh GP. Cognitive performance and cerebrohemodynamics associated with Persian Gulf Syndrome. *Toxicol Ind Health* 2001;17 (4):128-137.
3. Brimacombe M, Zhang Q, Lange G, Natelson BH. Immunological variables mediate cognitive dysfunction in Gulf War veterans but not civilians with chronic fatigue syndrome. *Neuroimmunomodulation* 2002;10(2):93-100.
4. Ronnback L, Hansson E. Does the astroglial network perform qualitative modifications of neuronal messages? In: Hansson E, Olsson T, Ronnback L, eds. *On Astrocytes and Glutamate Neurotransmission: New Waves in Brain Information Processing*. New York, N.Y.: Chapman & Hall; 1997:155-187.
5. Rimland B. The autism epidemic, vaccinations and mercury. *J Nutr Environ Med* 2000;10:261-266.
6. McGeer PL, McGeer EG. Local neuroinflammation and the progression of Alzheimer's disease. *J Neurovirol* 2002;8:529-538.
7. Wright JL, Merchant RE. Histopathological effects of intracerebral injections of human recombinant tumor necrosis factor-alpha in the rat. *Acta Neuropathol* (Berl) 1992;85:93-100.
8. Lawrence CB, Allan SM, Rothwell NJ. Interleukin-1ß and the interleukin receptor antagonist act in the stratum to modify excitotoxic brain damage in the rat. *Eur J Neurosci* 1998;10:1188-1195.
9. Mackenzie IA. Activated microglia in dementia with Lewy bodies. *Neurology* 2000;55:132-134.
10. Zhu X, Rottkamp CA, Hartzler A, et al. Activation of MKK6, an upstream activator of p38, in Alzheimer's disease. *J Neurochem* 2001;79:311-318.
11. Nicoll JA, Wilkinson D, Holmes C, et al. Neuropathology of human Alzheimer's disease after immunization with amyloid-beta peptide: a case report. *Nat Med* 2003;9:448-452.
12. Wood PL. Role of microglia in chronic neurodegenerative diseases. In: Wood PL, ed. *Neuroinflammation: Mechanisms and Management*. Totowa, N.J.: Humana Press; 2003:3-27.
13. Simmons RD, Willenborg DO. Direct injection of cytokines into the spinal cord causes autoimmune encephalomyelitis-like inflammation. *J Neurol Sci* 1990;100:37-42.
14. Jope RS, Zhang L, Song L. Peroxynitrite moderates the activation of p38 and extracellular regulated kinases in PC12 cells. *Arch Biochem Biophys* 2000;376:365-370.
15. Rothwell NJ, Luheshi GN. Interleukin 1 in the brain: biology, pathology, therapeutic target. *Trends Neurosci* 2000;23:618-625.
16. Murray CA, Lynch MA. Evidence that increased hippocampal expression of the cytokine interleukin-1ß is a common trigger for age- and stress-induced impairments in long-term potentiation. *J Neuroscience* 1998;18:2974-2981.
17. Vallieres L, Campbell IL, Gage FH, Sawchenko PE. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J Neurosci* 2002;22:486-492.
18. Neumann H, Schweigreiter R, Yamashita T, et al. Tumor necrosis factor inhibits neurite outgrowth and branching of hippocampal neurons by a Rho-dependent mechanism. *J Neurosci* 2002;22:854-862.

[19] Gillespie JS, Cavanagh HMA, Behan WMH, et al. Increased transcription of interleukin-6 in the brains of mice with chronic enterovirus infection. *J Gen Virol* 1993; 74: 741-743.

[20] Lynch WP, Robertson SJ, Portis JL. Induction of focal spongioform neurodegeneration in developmentally resistant mice by implantation of murine retrovirus-infected microglia. *J Virol* 1995;69:1408-1419.

[21] Heyes M, Saito K, Lacker A, et al. Sources of the neurotoxin quinolinic acid in the brain of HIV-1-infected patients and retrovirus-infected macaques. *FASEB J* 1998;12:881-896.

[22] Miniami M, Kuraishi Y, Satoh M. Effects of kainic acid on messenger RNA levels of IL-1beta, IL-6, TNF alpha and LIF in rat brain. *Biochem Biophys Res Commun* 1991;176:593-598.

[23] Blaylock RL. New Developments in the prevention and treatment of neurodegenerative diseases using nutraceuticals and metabolic stimulants. *J Am Nutr Assoc* 2002;5:15-32.

[24] Van Westerlaak MG, Jootsen EA, Gribnau AA, Cools AR, Bar PR. Chronic mitochondrial inhibition induces glutamate-mediated corticomotorneuron death in an organotypic culture model. *Exp Neurol* 2001;167:393-400.

[25] Weglicki WB, Phillips TM, et al. Magnesium deficiency elevates circulating levels of inflammatory cytokines and endothelin. *Mol Cell Biochem* 1992;110:169-173.

[26] Rockwell NJ. Cytokines-killer in the brain? *J Physiol* 1999;514:3-17.

[27] Park CH, Choi SH, Piao Y, et al. Glutamate and aspartate impair memory retention and damage hypothalamic neurons in adult mice. *Toxicol Lett* 2000;115:117-125.

[28] Mogenson GJ, Brudzynski SM, Wu M, Yang CR, Yim CCY. From motivation to action: regulation of limbic, nucleus accumbens, ventral pallidum, pedunculopontine nucleus circuitries involved in limbic-motor integration. In: Kalivas PW and Barnes CD, eds. *Limbic Circuits and Neuropsychiatry.* Boca Raton, Fla.: CRC Press; 1993:193-236.

[29] Blaylock RL. The central role of excitotoxicity in autism spectrum disorders. *J Am Nutr Assoc* 2003;6:7-19.

[30] Conner TJ, Leonard BE. Depression stress immunological activation: the role of cytokines in depressive disorders. *Life Sci* 1998;2:583-606.

[31] Poutiainen E, Hokkanen L, Niemi M-L, Forkkil M. Reversible cognitive decline during high-dose alpha-interferon treatment. *Pharmacol Biochem Behav* 1994;47:901-905.

[32] Denicoff KD, Rubinow DR, Papa MZ, et al. The neuropsychiatric effects of treatment with IL-2 and lymphokine-activated killer cells. *Ann Intern Med* 1987;107: 93-300.

[33] Turowski RC, Triozzi PL. Central nervous system toxicities of cytokine therapy. In: Plotnikoff NP, Faith RE, Murgo AJ, Good RA, eds. *Cytokines Stress and Immunity*. Boca Raton, Fla.: CRC Press; 1999:93-114.

[34] Renault PF, Hoofnagle JH, Park Y, et al. Psychiatric complications of long-term interferon-alpha therapy. *Arch Intern Med* 1987;147:1577-1580.

[35] Iaffaiolo RV, Fiorenza L, D'avino M, et al. Neurotoxic effects of long-term treatment with low-dose alpha 2b interferon. *Curr Ther Res* 1990;48:403-407.

[36] Meyers CA, Scheiel RS, Forman AD. Persistent neurotoxicity of systemically administered interferon-alpha. *Neurology* 1991;41:672-676.

[37] Adams F, Quesada JR, Gutterman JU. Neuropsychiatric manifestations of human leukocyte interferon therapy in patients with cancer. *JAMA* 1984;252:938-941.

[38] Kurzrock R, Quesada JR, Talpaz M, et al. Phase I study of multiple dose intramuscularly administered recombinant gamma interferon. *J Clin Oncol* 1986;4:1101-1109.

[39] Hu S, Sheng WS, Ehrlich LC, Peterson PK, Chao CC. Cytokine effects on glutamate uptake by human astrocytes. *Neuroimmunomodulation* 2000;7:153-159.

[40] Nicolson GL, Nasrally MY, Haier J, Pomfret J. High frequency of systemic mycoplasma infections in Gulf War veterans and civilians with amyotrophic lateral sclerosis (ALS). *J Clin Sci* 2002; 9:525-529.

[41] Moreno-Fuenmayor H, Borjas L, Arrieta A, Valera V, Socorro-Candanoza L. Plasma excitatory amino acids in autism. *Invest Clin* 1996;37:13-128.

[42] Wu J, Gao J, Zhao XN, Zhang ZX. Enhancing effects of Herpesvirus hominis on sodium glutamate neurotoxicity. *Zhongguo Yao Li Bao* 1994;15:161-164.

[43] Arai K, Matsuki N, Ikegaya Y, Nishiyama N. Deterioration of spatial learning performances in lipopolysaccharide-treated mice. *Jpn J Pharmacol* 2001;87:95-201.

[44] Broderick PA. Interleukin 1 alpha alters hippocampal serotonin and norepinephrine release during open-field behavior in Sprague-Dawley animals: differences from the Fawn-Hooded animal model of depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2002;26:1355-1372.

[45] Warren RP, Singh VK. Elevated serotonin levels in autism: association with the major histocompatibility complex. *Neuropsychobiology* 1996;34:72-75.

[46] Lellouch-Tubiana A, Fohlen M, Robain O, Rozenberg F. Immunocytochemical characterization of long-term persistent immune activation in human brain after herpes simplex encephalitis. *Neuropath Appl Neurobiol* 2000;26:285-294.

[47] Anderson T, Schultzberg M, Schwartz R, et al. NMDA-receptor antagonist prevents measles virus-induced neurodegeneration. *Eur J Neurosci* 1991;3:66-71.

[48] Booss J, Davis LE. Smallpox and smallpox vaccination: neurological implications. *Neurology* 2003;60:1241-1245.

[49] Rook GAW, Zumla A. Gulf War syndrome: is it due to a systemic shift in cytokine balance toward a Th2 profile? *Lancet* 1997;349:1831-1833.

[50] Everson MP, Kotler S, Blackburn WD. Stress and immune dysfunction in Gulf War veterans. *Ann NY Acad Sci* 1999;876:413-418.

[51] Asa PB, Wilson RB, Garry RF. Antibodies to squalene in recipients of anthrax vaccine. *Exp Mol Pathol* 2002;73:19-27.

[52] Warren RP, Singh VK, Averett RE, et al. Immunogenetic studies in autism and related disorders. *Mol Chem Neuropathol* 1996;28:77-81.

[53] Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain Behav Immunol* 1993;7(1):97-103.

[54] Malek-Ahmadi Cytokines and etiopathogenesis of pervasive developmental disorders. *Med Hypotheses* 2001;56:321-324.