

## REVIEW ARTICLE

A POSSIBLE CENTRAL MECHANISM IN  
AUTISM SPECTRUM DISORDERS, PART 1

Russell L. Blaylock, MD

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 immunexcitotoxic 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- $\alpha$ , can, via its receptor, interact with glutamate receptors to enhance the neurotoxic reaction. To describe this interaction I have coined the term *immunexcitotoxicity*, which is described in this article. (*Altern Ther Health Med.* 2008;14(6):46-53.)

**Russell L. Blaylock, MD, is a retired neurosurgeon and visiting professor of biology at Belhaven College, Jackson, Mississippi.**

*Editor's note: The following is part 1 of a 3-part series. Part 2 will appear in the Jan/Feb 2009 issue of Alternative Therapies in Health and Medicine.*

**A**utism 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.<sup>1,2</sup>

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.<sup>3,5</sup> 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.<sup>6,8</sup> 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.<sup>9-11</sup> 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.<sup>12,13</sup>

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.<sup>14</sup>

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.<sup>15,16</sup> 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.<sup>17</sup> John Olney in 1969 discovered that the food additive MSG could produce delayed neuron death when animals were fed the substance in higher concentrations.<sup>18</sup> 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.<sup>19</sup> Arrangement and expression of the various subtypes determines function. It is also known that glutamate receptors are not static but rather can be induced.<sup>20</sup>

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.<sup>21</sup> 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.<sup>22</sup>

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.<sup>23</sup> 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.<sup>24</sup> This explains the triggering of excitotoxic neuron destruction during hypoglycemia without glutamate elevation.<sup>25</sup>

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.<sup>26,28</sup> Glutamate has been shown to play a critical role in the development of the central nervous system.<sup>29,34</sup> Using glutamate receptor agonist ibotenate, Marret et al demonstrated that NMDA receptor excitotoxicity could arrest neural migration in a developing hamster brain.<sup>35</sup> They found intracortical and molecular layer heterotopias, 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.<sup>36</sup> 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.<sup>37,38</sup> Komuro and Rakic have demonstrated that fluctuations in intracellular calcium secondary to voltage-gated NMDA receptor channel activation con-

trols the speed of granule cells migration, with peaks speeding up migration and troughs slowing down migration.<sup>39</sup> 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.<sup>40</sup> 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.<sup>41</sup>

Prolonged behavioral abnormalities also have been demonstrated with glutamate exposure during development of the brain.<sup>42-44</sup> 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.<sup>45</sup> 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.<sup>46</sup> 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.<sup>47</sup> Most consistent in reported neuropathological studies of autism has been the finding of extensive loss of Purkinje cells in the cerebella of autistic patients.<sup>48,49</sup> 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.<sup>50-52</sup>

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.<sup>53</sup> 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, peroxy, and peroxyxynitrite.<sup>54</sup> Peroxyxynitrite is especially damaging to the mitochondria, resulting in enhanced reactive oxygen species (ROS) formation.<sup>55,56</sup> In addition, a number of lipid peroxidation products are produced during excitotoxicity, the most harmful being 4-hydroxynonenal.<sup>57,58</sup> Oxidative damage has been shown to occur in the developing brain and is exacerbated by glutamate excitotoxicity.<sup>59</sup>

## MICROGLIA, NEUROGENESIS, AND NEUROTOXICITY IN AUTISM SPECTRUM DISORDERS

Microglia represent the resident immune cell of the CNS.<sup>60</sup> 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 amoeboid morphology. Amoeboid 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  $\beta 2$ -integrins, leucocyte common antigen (LTA), immunoglobulin Fc $\gamma$  receptors, major histocompatibility complex class I (MHC-I) and MHC-II glycoproteins.<sup>61</sup>

Human microglia while in a ramified state constitutively express transcripts for messenger ribonucleic acid (mRNA) of interleukin (IL)-1 $\beta$ , IL-6, IL-8, IL-10, IL-12, IL-15, and tumor necrosis factor (TNF)- $\alpha$ , as well as macrophage inflammatory protein-1 (MIP-1), MIP-1 $\beta$ , and monocyte chemoattractant protein (MCP)-1. When activated, microglia express inflammatory cytokines (IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$ ), 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.<sup>62</sup> 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.<sup>63</sup>

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.<sup>64,65</sup> 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.<sup>66-70</sup>

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

In sheep, which reach term at 150 days, TNF- $\alpha$  appears first on embryonic day 30 (E30) and IL-1 $\beta$  at E35-45 in the cortical plate.<sup>73</sup> 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- $\alpha$  and IL-1 $\beta$  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.<sup>74</sup>

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.<sup>75</sup> 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.<sup>76</sup>

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.<sup>77</sup> Normally, the latter is tightly regulated. There is evidence that microglia play a significant role in neural migration and dendrite development.<sup>78,79</sup>

There also is evidence that excessive microglial activation can disrupt neurogenesis and neurodevelopment.<sup>80-83</sup> This disruption appears to involve inflammatory cytokines as well as glutamate excess.<sup>84,85</sup> Elevated levels of inflammatory cytokines and glutamate have been demonstrated in a number of studies of autistic children and adults.<sup>9-11</sup> In most of the amino acid studies, serum or blood glutamate levels were measured.<sup>86-88</sup> 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.<sup>89</sup>

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.<sup>38,90</sup> Neuropathologically, classic autistic patients have a reduced number of Purkinje cells in their cerebellum<sup>91</sup> and abnormally dense packing of neurons in the amygdala and hippocampus.<sup>92,93</sup>

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.<sup>94</sup> 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.<sup>95-97</sup>

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.<sup>96</sup> When activated acutely, microglia normally secrete both neurotoxic factors and neuroprotective factors.<sup>99</sup> Growing evidence indicates that chronic activation of microglia and astrocytes produces a state of predominant neurotoxicity.<sup>100-102</sup> 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.<sup>103-105</sup> One method by which cytokines and excitotoxins interact was shown by Floden et al, in which they demonstrated that TNF- $\alpha$  and glutamate synergistically stimulated neuronal iNOS expression with subsequent peroxynitrite production, which led to neuronal death.<sup>106</sup> Stimulation with either TNF- $\alpha$  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- $\alpha$  receptors TNFR1 and TNFR2 on the neuron, allowing cross-talk during stimulation. TNFR1 is predominantly neurotoxic, and TNFR2 is predominantly neuroprotective.<sup>107</sup> 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.<sup>108</sup> Similarly, it has been suggested that regional brain susceptibility to immune/excitotoxic injury is dependent on microglial population densities.<sup>109</sup>

Recently, Takeuchi et al demonstrated that TNF- $\alpha$  increases glutamate release from microglia by up-regulating glutaminase, the enzyme responsible for glutamate generation from glutamine.<sup>110</sup> In addition, they demonstrated that release of glutamate was not via glutamate transporters (EAATs) or the X<sub>c</sub><sup>-</sup> 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.<sup>47</sup> In this study, they performed immunocytochemistry, cytokine protein arrays, and enzyme-linked immunosorbent 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 anti-inflammatory 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 anti-inflammatory 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.<sup>111</sup> 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.<sup>112,113</sup>

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.<sup>114</sup>

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.<sup>115,116</sup> By accumulating in the brain, they may act as chronic immune stimulants.<sup>117,118</sup> Studies have shown that mercury preferentially accumulates in astrocytes.<sup>119</sup> 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.<sup>120</sup>

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).<sup>121-124</sup> 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.<sup>125</sup>

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

Churchill et al demonstrated that systemic IL-1 $\beta$  could increase brain IL-1 $\beta$  mRNA and that the effect was blocked by vagotomy.<sup>129</sup> Systemic IL-1 $\beta$  also increased TNF- $\alpha$  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- $\alpha$  was increased systemically, brain mRNA for TNF- $\alpha$  and IL-1 $\beta$  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-1 $\beta$  increased IL-6, IL-1 $\beta$ , and TNF- $\alpha$  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.<sup>130</sup>

It is also known that IL-1 $\beta$  activates neurons in the central nucleus of the amygdala<sup>131</sup> and that the same nucleus plays a major role in hypothalamic-pituitary adrenal (HPA) axis response to systemic immune stimulation.<sup>132</sup> Interestingly, some autistic children have been shown to have low corticoid secretion,<sup>133</sup> and others have hypersecretion of cortisol, thought to be related to stress.<sup>134</sup> This could indicate abnormalities in the glutamatergic-controlled HPA triggered by inflammation and excitotoxicity.<sup>135</sup> 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.<sup>136</sup>

Another important process is priming of microglia. Preexisting microglial activation has been shown to magnify neurodegenerative disease, with subsequent systemic immune stimulation.<sup>134,137</sup> 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-1 $\beta$  than when microglia were not primed.<sup>138</sup> IL-1 $\beta$ , being the central controlling cytokine for microglial activation, at these high levels would explain the chronic activation seen in the Vargas study. TNF- $\alpha$  levels increased to 1.7 times greater than unprimed state and IL-6, 3 times greater. Interestingly, no difference was found in TGF-1 $\beta$  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.<sup>139</sup> 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.<sup>140</sup>

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<sup>141,142</sup> or chronic intestinal infections with *Candida* or other dysbiotic organisms.<sup>143</sup>

Wakefield et al suggested a relationship between severe intestinal inflammation secondary to MMR vaccines and autism.<sup>144</sup> 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- $\alpha$  and CD3+IFN- $\gamma$  cytokine responses were significantly increased in children with autism spectrum disorder as compared to noninflamed control children.<sup>145</sup> 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.<sup>146</sup> 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.<sup>147</sup>

In a number of studies, reactions to commonly found colon bacterial organisms are seen to occur.<sup>146,148</sup> *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.<sup>149,150</sup> 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.<sup>151</sup> 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.<sup>152</sup> 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.<sup>153</sup> 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.<sup>154</sup> 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.<sup>155</sup> 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 tem-

poral 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.<sup>156</sup>

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.<sup>157</sup> 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.

#### REFERENCES

1. Rapin I. Autism. *N Engl J Med*. 1997;33(2):79-104.
2. Eigsti IM, Shapiro T. A systems neuroscience approach to autism: biological, cognitive, and clinical perspectives. *Ment Retard Dev Disabil Res Rev*. 2003;9(3):205-215.
3. Geier DA, Geier MR. A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. *Horm Res*. 2006;66(4):182-188.
4. Baron-Cohen S, Knickmeyer RC, Belmonte MK. Sex differences in the brain: implications for explaining autism. *Science*. 2005;310(5749):819-823.
5. Tordjman S, Ferrari P, Sulmont V, Duyme M, Roubertoux P. Androgenic activity in autism. *Am J Psychiatry*. 1997;154(11):1626-1627.
6. Kemper TL, Bauman M. Neuropathology of infantile autism. *J Neuropathol Exp Neurol*. 1998;57(7):645-652.
7. Bauman ML, Kemper TL. The neuropathology of autism spectrum disorders: what have we learned? *Novartis Found Symp*. 2003;251:112-122; discussion 122-128, 281-297.
8. Bailey A, Luthert P, Dean A, et al. A clinicopathological study of autism. *Brain*. 1998;121(Pt 5):889-905.
9. Cohlly HH, Panja A. Immunological findings in autism. *In Rev Neurobiol*. 2005;71:317-341.
10. Singh VK, Warren R, Averett R, Ghaziuddin M. Circulating autoantibodies to neuronal and glial filament proteins in autism. *Pediatr Neurol*. 1997;17(1):88-90.
11. Singh VK, Lin SX, Newell E, Nelson C. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. *J Biol Sci*. 2002;9(4):359-364.
12. Mutter J, Naumann J, Schneider R, Walach H, Haley B. Mercury and autism: accelerating evidence? *Neuro Endocrinol Lett*. 2005;26(5):439-446.
13. Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a novel form of mercury poisoning. *Med Hypotheses*. 2001;56(4):462-471.
14. Bailey A, Le Couteur A, Gottesman I, et al. Autism as a strongly genetic disorder: evidence from a British twin study. *Psychol Med*. 1995;25(1):63-77.
15. Blaylock RL. Chronic microglial activation and excitotoxicity secondary to excessive immune stimulation: possible factors in Gulf War Syndrome and autism. *J Am Phys Surg*. 2004;9(2):46-51.
16. Blaylock RL. Interaction of cytokines, excitotoxins, and reactive nitrogen and oxygen species in autism spectrum disorders. *J Am Nutraceutical Assoc*. 2003;6(4):21-35.
17. Lucas DR, Newhouse JP. The toxic effect of sodium L-glutamate in the inner layers of the retina. *AMA Arch Ophthalmol*. 1957;58(2):193-201.
18. Olney JW. Brain lesions, obesity, and other disturbances in mice treated with monosodium glutamate. *Science*. 1969;164(880):719-721.
19. Hollmann M, Heinemann S. Cloned glutamate receptors. *Annu Rev Neurosci*. 1994;17:31-108.
20. Wenthold RJ, Prybylowski K, Standley S, Sans N, Petralia RS. Trafficking of NMDA receptors. *Annu Rev Pharmacol Toxicol*. 2003;43:335-358.
21. Rigby M, Le Bourdellès B, Heavens RP, et al. The messenger RNAs for the N-methyl-D-aspartate receptor subunits show region-specific expression of different subunit composi-

- tion in the human brain. *Neuroscience*. 1996;73(2):429-447.
22. Beattie EC, Carroll RC, Yu X, et al. Regulation of AMPA receptor endocytosis by a signaling mechanism shared with LTD. *Nat Neurosci*. 2000;3(12):1291-1300.
  23. Schubert D, Piasecki D. Oxidative glutamate toxicity can be a component of the excitotoxicity cascade. *J Neurosci*. 2001;21(19):7455-7462.
  24. Novelli A, Reilly JA, Lysko PS, Henneberry RC. Glutamate becomes neurotoxic via the N-methyl-D-aspartate receptor when intracellular energy levels are reduced. *Brain Res*. 1988;451(1-2):205-212.
  25. Zeevalk GD, Nicklas WJ. Chemically induced hypoglycemia and anoxia: relationship to glutamate receptor-mediated toxicity in retina. *J Pharmacol Exp Ther*. 1990;253(3):1285-1292.
  26. Rothman S. Synaptic release of excitatory amino acid neurotransmitter mediates anoxic neuronal death. *J Neurosci*. 1984;4(7):1884-1891.
  27. Rothstein JD. Excitotoxicity hypothesis. *Neurology*. 1996;47(4 Suppl 2):S19-S25.
  28. Tekkök SB, Goldberg MP. Ampa/kainate receptor activation mediates hypoxic oligodendrocytes death and axonal injury in cerebral white matter. *J Neurosci*. 2001;21(12):4237-4248.
  29. Ghiani CA, Beltran-Parral Z, Sforza DM, et al. Genetic program of neuronal differentiation and growth induced by specific activation of NMDA receptors. *Neurochem Res*. 2007;32(2):363-376.
  30. Matsugami TR, Tanemura K, Mieda M, et al. Indispensability of the glutamate transporters GLAST and GLT1 to brain development. *Proc Natl Acad Sci U S A*. 2006;103(32):12161-12166.
  31. Schlett K. Glutamate as a modulator of embryonic and adult neurogenesis. *Curr Top Med Chem*. 2006;6(10):949-960.
  32. Bar-Peled O, Ben-Hur H, Biegon A, et al. Distribution of glutamate transporter subtypes during human brain development. *J Neurochem*. 1997;69(6):2571-2580.
  33. Komuro H, Rakic P. Modulation of neuronal migration by NMDA receptors. *Science*. 1993;260(5104):95-97.
  34. Rossi DJ, Slater NT. The developmental onset of NMDA receptor-channel activity during neuronal migration. *Neuropharmacology*. 1993;32(11):1239-1248.
  35. Marret S, Gressens P, Evrard P. Arrest of neuronal migration by excitatory amino acids in hamster developing brain. *Proc Natl Acad Sci U S A*. 1996;93(26):15463-15468.
  36. Komuro H, Yacubova E. Recent advances in cerebellar granule cell migration. *Cell Mol Life Sci*. 2003;60(6):1084-1098.
  37. Carper RA, Courchesne E. Inverse correlation between frontal lobe and cerebellum sizes in children with autism. *Brain*. 2000;123(Pt 4):836-844.
  38. Palmen SJ, van Engeland H, Hof PR, Schmitz C. Neuropathological findings in autism. *Brain*. 2004;127(Pt 12):2572-2583.
  39. Komuro H, Rakic P. Orchestration of neuronal migration by activity of ion channels, neurotransmitter receptors, and intracellular Ca<sup>2+</sup> fluctuations. *J Neurobiol*. 1998;37(1):110-130.
  40. Kumada T, Komuro H. Completion of neuronal migration regulated by loss of Ca<sup>2+</sup> transients. *Proc Natl Acad Sci U S A*. 2004;101(22):8479-8484.
  41. Olney JW. New insights and new issues in developmental neurotoxicology. *Neurotoxicology*. 2002;23(6):659-688.
  42. Wong PT, Neo LH, Teo WL, Feng H, Xue YD, Loke WH. Deficits in water escape performance and alterations in hippocampal cholinergic mechanisms associated with neonatal monosodium glutamate. *Pharmacol Biochem Behav*. 1997;57(1-2):383-388.
  43. Kubo T, Kohira R, Okano T, Ishikawa K. Neonatal glutamate can destroy the hippocampal CA1 structure and impair discrimination learning in rats. *Brain Res*. 1993;616(1-2):311-314.
  44. Frieder B, Grimm VE. Prenatal monosodium glutamate causes long lasting cholinergic and adrenergic changes in various brain regions. *J Neurochem*. 1987;48(5):1359-1365.
  45. Dubovicky M, Tokarev D, Skultetova I, Jezova D. Changes of exploratory behavior and its habituation in rats neonatally treated with monosodium glutamate. *Pharmacol Biochem Behav*. 1997;56(4):565-569.
  46. Hlinák Z, Grandalovicová D, Krejčí I. Behavioral deficits in adult rats neonatally treated with glutamate. *Neurotoxicol Teratol*. 2005;27(3):465-473.
  47. Vargas DL, Nascimbene C, Krissman C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Ann Neurol*. 2005;57(1):67-81.
  48. Bauman M, Kemper TL. Histoanatomic observations of the brain in early infantile autism. *Neurology*. 1985;35:866-874.
  49. Courchesne E. Brainstem, cerebellar and limbic neuroanatomical abnormalities in autism. *Curr Opin Neurobiol*. 1997;7(2):269-278.
  50. Allen G, Courchesne E. Differential effects of developmental cerebellar abnormality on cognitive and motor functions in the cerebellum: an fMRI study of autism. *Am J Psychiatry*. 2003;160(2):262-273.
  51. Schmahmann JD, Sherman JC. The cerebellar cognitive affective syndrome. *Brain*. 1998;121(Pt 4):551-579.
  52. Belmonte MK, Allen G, Beckel-Mitchener A, Boulanger LM, Carper RA, Webb SJ. Autism and abnormal development of brain connectivity. *J Neurosci*. 2004;24(42):9228-9231.
  53. Lewerenz J, Klein M, Methner A. Cooperative action of glutamate transporters and cystine/glutamate antiporter system Xc<sup>-</sup> protects from oxidative glutamate toxicity. *J Neurochem*. 2006;98(3):916-925.
  54. Bondy SC, Lee DK. Oxidative stress induced by glutamate receptor agonist. *Brain Res*. 1993;610(2):229-233.
  55. Lafon-Cazal M, Pietri S, Culcasi M, Bockaert J. NMDA-dependent superoxide production and neurotoxicity. *Nature*. 1993;364(6437):535-537.
  56. Hall ED, Detloff MR, Johnson K, Kupina NC. Peroxynitrite-mediated protein nitration and lipid peroxidation in a mouse model of traumatic brain injury. *J Neurotrauma*. 2004;21(1):9-20.
  57. McCracken E, Valeriani V, Simpson C, Jover T, McCulloch J, Dewar D. The lipid peroxidation-by-product 4-hydroxynonenal is toxic to axons and oligodendrocytes. *J Cereb Blood Flow Metab*. 2000;20(11):1529-1536.
  58. McCracken E, Graham DI, Nilsen M, Stewart J, Nicoll JA, Horsburgh K. 4-Hydroxynonenal immunoreactivity is increased in human hippocampus after global ischemia. *Brain Pathol*. 2001;11(4):414-421.
  59. Verity MA. Oxidative damage and repair in the developing nervous system. *Neurotoxicology*. 1994;15(1):81-91.
  60. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*. 2007;8(1):57-69.
  61. Kim SU, de Vellis J. Microglia in health and disease. *J Neurosci Res*. 2005;81(3):302-313.
  62. Boddles AM, Barger SW. Secreted beta-amyloid precursor protein activates microglia via JNK and p38-MAPK. *Neurobiol Aging*. 2005;26(1):9-16.
  63. Kyriakis JM, Banerjee P, Nikolakaki E, et al. The stress-activated protein kinase subfamily of c-Jun kinases. *Nature*. 1994;369(6476):156-160.
  64. Piani D, Frei K, Do KQ, Cuénod M, Fontana A. Murine brain macrophages induce NMDA receptor mediated neurotoxicity in vitro by secreting glutamate. *Neurosci Lett*. 1991;133(2):159-162.
  65. Guillemin GJ, Williams KR, Smith DG, Smythe GA, Croitoru-Lamourey J, Brew BJ. Quinolinic acid in the pathogenesis of Alzheimer's disease. *Adv Exp Med Biol*. 2003;527:167-176.
  66. Farber K, Kettenmann H. Physiology of microglial cells. *Brain Res Brain Res Rev*. 2005;48(2):133-143.
  67. Noda M, Nakanishi H, Nabekura J, Akaike N. AMPA-kainate subtype of glutamate receptor in rat cerebral microglia. *J Neurosci*. 2000;20(1):251-258.
  68. D'Aversa TG, Yu KO, Berman JW. Expression of chemokines by human fetal microglia after treatment with the human immunodeficiency virus type 1 protein Tat. *J Neurovirol*. 2004;10(2):86-97.
  69. Kim MO, Si Q, Zhou JN, et al. Interferon-beta activates multiple signaling cascades in primary human microglia. *J Neurochem*. 2002;81(6):1361-1371.
  70. Godbout JP, Berg BM, Kelley KW, Johnson RW. alpha-Tocopherol reduces lipopolysaccharide-induced peroxide radical formation and interleukin-6 secretion in primary murine microglia and in brain. *J Neuroimmunol*. 2004;149(1-2):101-109.
  71. Lee SC, Liv W, Dickson DW, Brosnan CF, Berman JW. Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1beta. *J Immunol*. 1993;150(7):2659-2667.
  72. Basu A, Kradky JK, Levison SW. Interleukin-1: a master regulator of neuroinflammation. *J Neurosci Res*. 2004;78(2):151-156.
  73. Dziegielewska KM, Møller JE, Potter AM, Ek J, Lane MA, Saunders NR. Acute-phase cytokines IL-1beta and TNF-alpha in brain development. *Cell Tissue Res*. 2000;299(3):335-345.
  74. Sargent IL. Maternal and fetal immune responses during pregnancy. *Exp Clin Immunogenet*. 1993;10(2):85-102.
  75. Ashwell K. Microglia and cell death in developing mouse cerebellum. *Brain Res Dev Brain Res*. 1990;55(2):219-230.
  76. Bessis A, Béchade C, Bernard D, Roumier A. Microglial control of neuronal death and synaptic properties. *Glia*. 2007;55(3):233-238.
  77. Nakajima K, Kohsaka S. Neuroprotective roles of microglia in the central nervous system. In: Streit WJ, ed. *Microglia in the Regenerating and Degrenerating Central Nervous System*. New York: Springer; 2001: 188-208.
  78. Aarum J, Sandberg K, Budd Haerberlein SL, Persson MA. Migration and differentiation of neural precursor cells can be directed by microglia. *Proc Natl Acad Sci U S A*. 2003;100(26):15983-15988.
  79. Ghiani CA, Beltran-Parral Z, Sforza DM, et al. Genetic program of neuronal differentiation and growth induced by specific activation of NMDA receptors. *Neurochem Res*. 2007;32(2):363-376.
  80. Ekdahl CT, Claassen JH, Bonde S, Kokaia Z, Lindvall O. Inflammation is detrimental for neurogenesis in adult brain. *Proc Natl Acad Sci U S A*. 2003;100(23):13632-13635.
  81. Monje ML, Toda H, Palmer TD. Inflammatory blockade restores adult hippocampal neurogenesis. *Science*. 2003;302(5651):1760-1765.
  82. Vallières L, Campbell, Gage FH, Sawchenko PE. Reduced hippocampal neurogenesis in adult transgenic mice with chronic astrocytic production of interleukin-6. *J Neurosci*. 2002;22(2):486-492.
  83. Suzuki M, Nelson AD, Eickstaedt JB, Wallace K, Wright LS, Svendsen CN. Glutamate enhances proliferation and neurogenesis in human neural progenitor cell cultures derived from fetal cortex. *Eur J Neurosci*. 2006;24(3):645-653.
  84. Chao CC, Hu S. Tumor necrosis factor-alpha potentiates glutamate neurotoxicity in human fetal cell cultures. *Dev Neurosci*. 1994;16(3-4):172-179.
  85. Luk KC, Kennedy TE, Sadikot AF. Glutamate promotes proliferation of striatal neuronal progenitors by an NMDA receptor-mediated mechanism. *J Neurosci*. 2003;23(6):2239-2250.
  86. Shinohe A, Hashimoto K, Nakamura K, et al. Increased serum levels of glutamate in adult patients with autism. *Prog Neuropsychopharmacol Biol Psychiatry*. 2006;30(8):1472-1477.
  87. Aldred S, Moore KM, Fitzgerald M, Waring RH. Plasma amino acid levels in children with autism and their families. *J Autism Dev Disord*. 2003;33(1):93-97.
  88. Moreno-Fuenmayor H, Borjas L, Arrieta A, Valera V, Socorro-Candanoza L. Plasma excitatory amino acids in autism. *Invest Clin*. 1996;37(2):113-128.
  89. Hamberger A, Gillberg C, Palm A, Hagberg B. Elevated CSF glutamate in Rett syndrome. *Neuropediatrics*. 1992;23(4):212-213.
  90. Saitoh O, Courchesne E. Magnetic resonance imaging study of the brain in autism. *Psychiatry Clin Neurosci*. 1998 Dec;52 Suppl:S219-S222.
  91. Ritvo ER, Freeman BJ, Scheibel AB, et al. Lower Purkinje cell counts in cerebella of four autistic subjects: initial findings of the UCLA-NSAC Autopsy Research Report. *Am J Psychiatry*. 1986;143(7):862-866.
  92. Bauman M, Kemper TL. Histoanatomic observations of the brain in early infantile autism. *Neurology*. 1985;35(6):866-874.
  93. Raymond GV, Bauman ML, Kemper TL. Hippocampus in autism: a Golgi analysis. *Acta Neuropathol (Berl)*. 1996;91(1):117-119.
  94. Courchesne E. Neuroanatomic imaging in autism. *Pediatrics*. 1991;87(5 Pt 2):781-790.

95. Schlett K. Glutamate as a modulator of embryonic and adult neurogenesis. *Curr Top Med Chem*. 2006;6(10):949-960.
96. Tancredi V, D'Antuono M, Café C, et al. The inhibitory effects of interleukin-6 on synaptic plasticity in the rat hippocampus are associated with inhibition of mitogen-activated protein kinase ERK. *Neurochem*. 2000;75(2):634-643.
97. Nacher J, McEwen BS. The role of N-methyl-D-aspartate receptors in neurogenesis. *Hippocampus*. 2006;16(3):267-270.
98. Lee SC, Liu W, Dickson DW, Brosnan CF, Berman JW. Cytokine production by human fetal microglia and astrocytes. Differential induction by lipopolysaccharide and IL-1beta. *J Immunol*. 1993;150(7):2659-2667.
99. Rothwell NJ, Luheshi GN. Interleukin 1 in the brain: biology, pathology and therapeutic target. *Trends Neurosci*. 2000;23(12):618-625.
100. Vallières 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(2):486-492.
101. Basu A, Krady JK, O'Malley M, Styren SD, DeKosky ST, Levison SW. The type 1 interleukin-1 receptor is essential for the efficient activation of microglia and the induction of multiple proinflammatory mediators in response to brain injury. *J Neurosci*. 2002;22(14):6071-6082.
102. Rothwell NJ. Annual review prize lecture cytokines—killers in the brain? *J Physiol*. 1999;514 (Pt 1):3-17.
103. Willard LB, Hauss-Wegryniak B, Danysz W, Wenk GL. The cytotoxicity of chronic neuroinflammation upon basal forebrain cholinergic neurons of rats can be attenuated by glutamatergic antagonism or cyclooxygenase-2 inhibition. *Exp Brain Res*. 2000;134(1):58-65.
104. Barger SW, Basile AS. Activation of microglia by secreted amyloid precursor protein evokes release of glutamate by cystine exchange and attenuates synaptic function. *J Neurochem*. 2001;76(3):846-854.
105. Hegg CC, Thayer SA. Monocytic cells secrete factors that evoke excitatory synaptic activity in rat hippocampal cultures. *Eur J Pharmacol*. 1999;385(2-3):231-237.
106. Floden AM, Li S, Combs CK. Beta-amyloid-stimulated microglia induce neuronal death via synergistic stimulation of tumor necrosis factor alpha and NMDA receptors. *J Neurosci*. 2005;25(10):2566-2575.
107. Yang L, Lindholm K, Konishi Y, Li R, Shen Y. Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuronal death and survival through different signal transduction pathways. *J Neurosci*. 2002;22(8):3025-3032.
108. Sinor JD, Du S, Venneti S, et al. NMDA and glutamate evoke excitotoxicity at distinct cellular locations in rat cortical neurons in vitro. *J Neurosci*. 2000;20(23):8831-8837.
109. Kim WG, Mohney RP, Wilson B, Jeohn GH, Liu B, Hong JS. Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J Neurosci*. 2000;20(16):6309-6316.
110. Takeuchi H, Jin S, Wang J, et al. Tumor necrosis factor-alpha induces neurotoxicity via glutamate release from hemichannels of activated microglia in an autocrine manner. *J Biol Chem*. 2006;281(30):21362-21368.
111. Leonard EJ, Yoshimura T. Human monocyte chemoattractant protein-1 (MCP-1). *Immunol Today*. 1990;11(3):97-101.
112. Saliba E, Henrot A. Inflammatory mediators and neonatal brain damage. *Biol Neonate*. 2001;79(3-4):224-227.
113. Gadiant RA, Otten U. Expression of interleukin-6 (IL-6) and interleukin-6 receptor (IL-6R) mRNAs in rat brain during postnatal development. *Brain Res*. 1994;637(1-2):10-14.
114. Perry VH, Newman TA, Cunningham C. The impact of systemic infection on the progression of neurodegenerative disease. *Nat Rev Neurosci*. 2003;4(2):103-112.
115. Charleston JS, Body RL, Bolender RP, Mottet NK, Vaher ME, Burbacher TM. Changes in the number of astrocytes and microglia in the thalamus of the monkey *Macaca fascicularis* following long-term subclinical methylmercury exposure. *Neurotoxicology*. 1996;17(1):127-138.
116. Campbell A, Becaria A, Lahiri DK, Sharman K, Bondy SC. Chronic exposure to aluminum in drinking water increases inflammatory parameters selectively in the brain. *J Neurosci Res*. 2004;75(4):565-572.
117. Shirabe T, Irie K, Uchida M. Autopsy case of aluminum encephalopathy. *Neuropathology*. 2002;22(3):206-210.
118. Charleston JS, Bolender RP, Mottet NK, Body RL, Vaher ME, Burbacher TM. Increases in the number of reactive glia in the visual cortex of *Macaca fascicularis* following subclinical long-term methyl mercury exposure. *Toxicol Appl Pharmacol*. 1994;129(2):196-206.
119. Aschner M, Rising L, Mullaney KJ. Differential sensitivity of neonatal rat astrocyte cultures to mercuric chloride (MC) and methylmercury (MeHg): studies on K+ and amino acid transport and metallothionein (MT) induction. *Neurotoxicology*. 1996;17(1):107-116.
120. Davoust N, Vuillat C, Cavillon G, et al. Bone marrow CD34+/B220+ progenitors target the inflamed brain and display in vitro differentiation potential toward microglia. *FASEB J*. 2006;20(12):2081-2092.
121. Rockwood K, Cosway S, Carver D, Jarrett P, Stadnyk K, Fisk J. The risk of dementia after delirium. *Age Aging*. 1999;28(6):551-556.
122. Holmes C, El-Okil M, Williams AL, Cunningham C, Wilcockson D, Perry VH. Systemic infection, interleukin 1beta, and cognitive decline in Alzheimer's disease. *J Neurol Neurosurg Psychiatry*. 2003;74(6):788-789.
123. Perry VH, Newman TA, Cunningham C. Impact of systemic infection on the progression of neurodegenerative disease. *Nat Rev Neurosci*. 2003;4(2):103-112.
124. Sibley WA, Bamford CR, Clark K. Clinical viral infections and multiple sclerosis. *Lancet*. 1985;1(8441):1313-1315.
125. Vereker E, Campbell V, Roche E, McEntee E, Lynch MA. Lipopolysaccharide inhibits long term potentiation in rat dentate gyrus by activating caspase-1. *J Biol Chem*. 2000;275(34):26252-26258.
126. Nguyen MD, D'Aigle T, Gowing G, Julian J, Rivest S. Exacerbation of motor neuron disease by chronic stimulation of innate immunity in a mouse model of amyotrophic lateral sclerosis. *J Neurosci*. 2004;24(6):1340-1349.
127. Layé S, Parnet P, Goujon E, Dantzer R. Peripheral administration of liposaccharide induces the expression of cytokine transcripts in brain and pituitary of mice. *Brain Res Mol Brain Res*. 1994;27(1):157-162.
128. Combrinck MI, Perry VH, Cunningham C. Peripheral infection evokes exaggerated sickness behavior in pre-clinical murine prion disease. *Neuroscience*. 2002;112(1):7-11.
129. Churchill L, Taishi P, Wang M, et al. Brain distribution of cytokine mRNA induced by systemic administration of interleukin-1beta or tumor necrosis factor alpha. *Brain Res*. 2006;1120(1):64-73.
130. Juranek J, Filippek PA, Berenji GR, Modahl C, Osann K, Spence MA. Association between amygdala volume and anxiety level: magnetic resonance imaging (MRI) study in autistic children. *J Child Neurol*. 2006;21(12):1051-1058.
131. Buller KM, Day TA. Systemic administration of interleukin-1beta activates select populations of central amygdala afferents. *J Comp Neurol*. 2002;452(3):288-296.
132. Xu Y, Day TA, Buller KM. The central amygdala modulates hypothalamic-pituitary-adrenal axis responses to systemic interleukin-1beta administration. *Neuroscience*. 1999;94(1):175-183.
133. Curin JM, Terzic J, Petkovic ZB, Zekan L, Terzic IM, Sunjara IM. Lower cortisol and higher ACTH levels in individuals with autism. *J Autism Dev Disord*. 2003;33(4):443-448.
134. Jansen LM, Gispens-de Wied CC, van der Gaag RJ, van Engeland H. Differentiation between autism and multiple complex developmental disorder in response to psychosocial stress. *Neuropsychopharmacology*. 2003;28(3):582-590.
135. Wittmann G, Lechan RM, Liposits Z, Fekete C. Glutamatergic innervation of corticotropin-releasing hormone- and thyrotropin-releasing hormone-synthesizing neurons in the hypothalamic paraventricular nucleus of the rat. *Brain Res*. 2005;1039(1-2):53-62.
136. Kheir-Eldin AA, Motawi TK, Gad MZ, Abd-ElGawad HM. Protective effect of vitamin E, beta-carotene and N-acetylcysteine from the brain oxidative stress induced in rats by lipopolysaccharide. *Int J Biochem Cell Biol*. 2001;33(5):475-482.
137. Combrinck MI, Perry VH, Cunningham C. Peripheral infection evokes exaggerated sickness behavior in pre-clinical murine prion disease. *Neuroscience*. 2002;112(1):7-11.
138. Cunningham C, Deacon R, Wells H, et al. Synaptic changes characterize early behavioral signs in the ME7 model of murine prion disease. *Eur J Neurosci*. 2003;17(10):2147-2155.
139. Katayama Y, Hotta H, Nishimura A, Tatsuno Y, Homma M. Detection of measles virus nucleoprotein mRNA in autopsied brain tissues. *J Gen Virol*. 1995;76(Pt 2):3201-3204.
140. Authier FJ, Cherin P, Creange A, et al. Central nervous system disease in patients with macrophagic myofasciitis. *Brain*. 2001;124(Pt 5):974-983.
141. Lucarelli S, Frediani T, Zingoni AM, et al. Food allergy and infantile autism. *Panminerva Med*. 1995;37(3):137-141.
142. O'Banion D, Armstrong B, Cummings RA, Stange J. Disruptive behavior: a dietary approach. *J Autism Child Schizophr*. 1978;8(3):325-337.
143. Kidd PM. Autism, an extreme challenge to integrative medicine. Part II: medical management. *Altern Med Rev*. 2002;7(6):472-499.
144. Wakefield AJ, Murch SH, Anthony A, et al. Ileal-lymphoid-nodular hyperplasia, non-specific colitis, and pervasive developmental disorder in children. *Lancet*. 1998;351(9103):637-641.
145. Ashwood P, Wakefield AJ. Immune activation of peripheral blood and mucosal CD3+ lymphocyte cytokine profiles in children with autism and gastrointestinal symptoms. *J Neuroimmunol*. 2006;173(1-2):126-134.
146. Vojdani A, Campbell AW, Anyanwu E, Khashanian A, Bock K, Vojdani E. Antibodies to neuron-specific antigens in children with autism: possible cross-reaction with encephalitogenic proteins from milk, *Chlamydia pneumoniae* and *Streptococcus group A*. *J Neuroimmunol*. 2002;129(1-2):168-177.
147. Vojdani A, O'Bryan T, Green JA, et al. Immune response to dietary proteins, gliadin and cerebellar peptides in children with autism. *Nutr Neurosci*. 2004;7:151-161.
148. Jyonouchi H, Sun S, Itokazu N. Innate immunity associated with inflammatory responses and cytokine production against common dietary proteins in patients with autism spectrum disorder. *Neuropsychobiology*. 2002;46(2):76-84.
149. Hida S, Miura NN, Adachi Y, Ohno N. Effect of *Candida albicans* cell wall glucan as adjuvant for induction of autoimmune arthritis in mice. *J Autoimmun*. 2005;25(2):93-101.
150. Hida S, Nagi-Miura N, Adachi Y, Ohno N. Beta-glucan derived from zymosan acts as an adjuvant for collagen-induced arthritis. *Microbiol Immunol*. 2006;50(6):453-461.
151. Black C, Kaye JA, Jick H. Relation of childhood gastrointestinal disorders to autism: nested case-control study using data from the UK General Practice Research Database. *BMJ*. 2002;325(7361):419-421.
152. Luostarinen LK, Collin PO, Peräaho MJ, Mäki MJ, Pirttilä TA. Coeliac disease in patients with cerebellar ataxia of unknown origin. *Ann Med*. 2001;33(6):445-449.
153. Bürk K, Bösch S, Müller CA, et al. Sporadic cerebellar ataxia associated with gluten sensitivity. *Brain*. 2001;124(Pt 5):1013-1019.
154. Hadjivassiliou M, Grünewald R, Sharrack B, et al. Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. *Brain*. 2003;126(Pt 3):685-691.
155. Hu WT, Murray JA, Greenaway MC, Parisi JE, Josephs KA. Cognitive impairment and celiac disease. *Arch Neurol*. 2006;63(10):1440-1446.
156. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*. 2007;8(1):57-69.
157. Hadjivassiliou M, Boscolo S, Davies-Jones GA, et al. The humoral response in pathogenesis of gluten ataxia. *Neurology*. 2002;58(8):1221-1226.