Neuroinflammation and Autism

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The incidence of autism spectrum disorder (ASD) has increased significantly in the past decades, now affecting 1 out of 68 children in USA. The complexity of this disorder and the unclear mechanisms have hindered the development of an effective therapeutic regimen. Recent studies have suggested that neuro-inflammation plays an important role in the pathogenesis of ASD. A literature review was conducted to examine the evidence of various central immune processes involved in ASD. Conventional and novel medications for ASD treatment were summarized.

Key Words: *autism, neuro-inflammation, mast cell, microglia, astrocyte, medication*

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INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by deficits in social interaction, social communication, restricted and repetitive interests, and behavioral patterns.[1] Symptoms of affected children usually manifest by the age of 3. The occurrence of ASD has increased dramatically in the past decade to 1 in 68 in 2010 in the USA according to the recent report from the Centers for Disease Control and Prevention (CDC).[2] The pathogenesis for ASD is unclear, and there is no effective treatment. Several epigenetic triggers have been identified, including trauma, adverse reactions to vaccination, infection, and allergies. Neuro-inflammation characterized by microglia/astrocyte activation and brain inflammatory cytokine production have been documented in several post-mortem studies as well as in biomarker studies. Overactivation of microglia and astrocytes has been found in several brain regions during autopsies of autistic brains.[3-5] Several studies have shown elevated inflammatory cytokines (such as interleukin (IL)-1, IL-6, IL8, IL12, tumor necrosis factor (TNF)-α) in serum, plasma, and cerebral spinal fluid(CSF) in children with autism.[4,6,7] It has been suggested that abnormal activation of neuro-inflammation in ASD begins in the perinatal period. Pre-natal or early post-natal inflammation and infection have been shown to lead to neurodevelopmental dysfunction in ASD. A Danish study investigated about 689,196 births over a decade. The result shows that mothers with rheumatoid arthritis increased the risk of children of developing autism by 80 percent.[8] An animal study showed that immune activated pregnant mice via injecting the viral mimic poly (I:C) produced newborn mice with autistic-like behavior through the collateral damage.[9,10] Therefore, growing evidence implies that prolonged abnormal activation in brain inflammation contributes to the pathogenesis of autism. These new findings are leading to the development of novel therapeutic therapies.

MAST CELLS ACTIVATION AND AUTISM

Mast cells are the key players in the inflammatory response and present in all tissues. In the brain, they are mostly found in the diencephalon[11] and generate pro- and anti-inflammatory cytokines upon activation. Mast cell activation can be triggered by both allergic and non-allergic reactions. Mast cells also induce neutrophil infiltration and modulate microglial activation, mediating the neuro-inflammatory process.[12] The fact that ASD children have common immune disorders implicates overactivation of mast cells in ASD[13] ASD children have more allergic-like reactions. Several inflammatory diseases increase the chance of developing autism, for instance, it was reported that celiac disease increases the risk of ASD by 350%.[8]

Evidence suggests that mast cell activation contributes to a malfunction of blood brain barrier (BBB) [14,15] in ASD, as evidenced by serum antibodies that attack fetal brain tissues.[16-19] Several vasoactive peptides that activate mast cells have been identified. Neurotensin (NT) stimulates mast cells in various tissues, including skin, peritoneum, and brain, resulting in increased vascular permeability.[20] NT is isolated in brain and gut tissues. In the brain, mast cell activation by NT leads to secretion of vascular endothelial growth factor (VEGF),[21] which contributes to BBB damage. Corticotropin-releasing hormone (CRH) was found to act synergistically with NT to stimulate mast cells.[22] A recent study reports that NT is increased in the serum of children with autism, implicating the role of NT in autism pathogenesis.[23] In addition, cytokine IL-33 has also been implicated to stimulate mast cells.[24]

MICROGLIAL ACTIVATION IN AUTISM

Microglia are resident macrophages of the brain and play a critical role in the immune system of the brain. In addition, microglia regulate synaptogenesis[25] and neurogenesis.[26] Microglia are activated during brain insults, including ischemia, trauma, and infection. Activated microglia secrete pro-inflammatory cytokines, including IL-1β, TNF-α , IL-6, and the inducible form of nitric oxide synthase (iNOS).[27] Activation of microglia cells is required for normal brain defense mechanisms. However, overactivation of microglia leads to chronic neuroinflammation, which is neurodestructive.

In autism, increasing evidence suggests chronic activation of microglia. Post-mortem brain examination has revealed abnormally activated microglia and astrocytes in multiple brain regions.[4,5,28] Morgan et al[5] found microglial activation and increased microglial density in the dorsolateral prefrontal cortex of brains with autism (n=13) as compared to controls (n=9). It is of interest that microglial activation presents in early age. Two of three autism cases under the age of 6 had markedly microglial activation, suggesting microglial activation may play an important role in the pathogenesis of autism.[5] In another post-mortem study, Vargas and colleagues reported that microglial activation was widespread in the cortical and subcortical regions, including the cerebellum, midfrontal and cingulate gyrus.[4] Tetreaulte et al (2012) reported microglial activation in the fronto-insular and visual cortices in subjects with autism as compared to matched controls. Because the two regions are anatomically distinct, it is speculated that the density of microglia in brains with autisms might be higher throughout the cerebral cortex.[28] Furthermore, a recent case-controlled study using positron emission tomography (PET) revealed overactivation of microglia in multiple brain regions.[29] In this PET study, 20 men with ASD (age range 18-31) and 20 age-matched healthy men underwent 3-dimensional magnetic resonance images (MRIs) prior to PET measurement. The activated microglial density in the brain was measured using radiotracer for microglia – [11C](R)-PK11195. The radiotracer binding potential was significantly increased in multiple brain regions (such as the cerebellum, midbrain, pons, and fusiform gyri) in subjects with ASD compared with controls. Among the above brain regions, the cerebellum showed the most prominent hyperactivation in those with ASD.[29] The cytokine profile from brain tissue and CSF also suggests neuroglial activation in subjects with autism. Vargas et al examined cytokine levels in fresh-frozen tissues from seven autistic patients and CSF from six living autistic patients. In the brain tissues, macrophage chemoattractant protein (MCP)-1 was increased in the anterior cingulated gyrus. Tumor growth factor (TGF)-beta 1, which is derived from microglia and astroglia, was significantly increased in the middle frontal gyrus in autistic patients. Protein array analysis of the CSF showed significant elevation of MCP-1 in autistic subjects.[4] Several other studies also report elevation of proinflammatory cytokines in the CSF or brain tissue of autistic subjects. Chez et at showed that TNF-α was increased in the CSF of autistic patients.[7] A study conducted by Li et al showed that TNF-α, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-8, and IFN-γ were significantly elevated in the brain tissue of autistic patients as compared to controls.[30]

It has been suggested that overactivation of microglia in those with ASD most likely begins during the perinatal period and lasts until adulthood. For example, terbutaline, which is used to arrest preterm labor, has been associated with an increased risk for autism[31] and cognitive deficit.[32] Animals treated with terbutaline on post-natal days 2 and 5 showed marked microglial activation associated with autistic-like behavior. On the other hand, animals treated with terbutaline on post-natal days 11 to 14 did not have any of the effects.[33]

ASTROCYTE ACTIVATION AND AUTISM

Astrocytes are the most abundant cell type in the human brain. They play important roles in diverse physiological functions, including repairing brain tissue after injury, supporting endothelial cells lining the BBB, maintaining extracellular ion balance, and transmitting nutrients to neurons.[34] Astrocytes also play critical roles in synaptogenesis during development. However, extended activation of astrocytes contributes to gliosis and brain damage. Several studies have found increased levels of astrocyte markers (glial fibrillary acidic protein, GFAP) in the CSF in children with autism, suggesting reactive astrogliosis in the central nervous system (CNS).[35,36] Consistently, GFAP was also elevated in multiple post-mortem brain regions in children with autism as compared to matched controls. Laurence and Fatemi reported that GFAP was elevated in frontal, parietal, and cerebellar cortices in autistic specimens as compared to controls.[37] In addition, other astrocyte markers (aquaporin 4 and connexin 43) are also increased in autistic brains as compared to healthy controls.[38]

NEUROINFLAMMATION AND UNDER-CONNECTI-VITY

Many studies have suggested significant disruption of connectivity in autistic brains. One study found that the severity of connectivity abnormality correlates with the severity of the ASD symptoms.[39,40] Functional magnetic

resonance imaging (fMRI) revealed lower connectivity between the frontal and parietal-occipital regions.[41] Other studies using fMRI showed reduced functional connectivity between anterior and posterior insula, striatal subregions, and limbic cortex in ASD as compared to controls.[42] In a study using whole-brain MRI morphometric surveys of asymmetry in children with high-functioning autism and with developmental language disorder (DLD), Herbert et al reported that ASD and DLD showed more asymmetry at the level of cortical parcellation units, especially in the higher-order association areas.[43] In addition, in a different study, Hertbert et al also reported that ASD and DLD had increased total brain and white matter volumes without an increase in the size of the corpus callosum.[44] Consistently, Boer-Mediddo et al found that ASD callosums were small when adjusted for increased ASD cerebral volume. More severely affected ASD subjects have greater proportional callosum reduction than developmental disorder.[39] Taken together, those data suggest that ASD and DLD have connectivity abnormalities in higher-order association areas.

Decreased functional connectivity between cortical regions has been demonstrated in wide variety of neurodevelopmental disorders, including autism,[45,46] schizophrenia,[47] epilepsy,[48] obsessive compulsive disorder.[49] Accumulating evidence suggests that deficit in synaptic maturation may contribute to the pathophysiology of mental illness.[50] Microglia have been shown to play an important role in the elimination of synapses during brain development, so-called "pruning".[51] It was shown that microglia actively engulf synaptic material.[51] Mice with reduction of microglia, due to lack of chemokine Cx3cr1 during the early postnatal period developed excess of dendritic spines, immature synpases and immature brain circuitry.[25] Furthermore, the mice with deficiency in synaptic pruning have weak synaptic transmission, decreased brain connectivity and deficits in social interaction and other autistic behaviors.[52]

Furthermore, chronic neuroinflammation can contribute to progressive neurodestruction, leading to worsening of ASD.[53] Cytokines released by activated microglia and astrocytes are accumulated during the prolonged neuroglial activation.[54] A abnormally high level of cytokines has been shown to result in neuronal death, which leads to loss of connectivity.[55]

DRUG TREATMENT

Pharmacological management in ASD has traditionally targeted psycho-behavioral symptoms including anxiety, hyperactivity, aggression, irritability, and stereotypical behaviors.[56]

Antidepressants are also used in treating behavioral symptom in ASD. One clinical trial has reported improvement in obsessive-compulsive symptoms with fluoxetine.[57] There are also studies suggesting beneficial effects of other serotonin reuptake inhibitors (SSRI) such as citalopram[58] and escitalopram.[59] However, meta-analysis and Cochrane review did not reveal the efficacy.[60] Moreover, one study suggests that citalopram induced more adverse events including impulseness, decreased concentration and hyperactivity.[61] Therefore, the effects of antidepressants are unclear and need further study.

Many studies have investigated the efficacy of antipsychotics in ASD treatment for behavioral management. Typical antipsychotics such as haloperidol have been used much less due to the side effects, including extrapyramidal symptoms (EPS).[62] Atypical antipsychotics have been used more often for behavioral dysfunction, such as steorotypies, irritability, hyperactivity, and aggression.[63] Risperidone[64] and aripiprazole[65] are the two FDA-approved atypical anti-psychotics for treatment of behavioral symptoms in children and adolescents with ASD. Both drugs have side effects, including weight gain and sedation.[66,67] Other drugs such as neurostimulants (methylphenidate) have been used to treat ASD. Several clinical trials report beneficial effects, even though the adverse events were more common.[68-70] Melatonin is used to treat sleep disorders. Studies have suggested the potential benefit in treating insomnia in ASD;[71-73] however, larger trials are needed to confirm the results. Some studies report that oxytocin infusion reduced repetitive behaviors.[74,75] Two studies showed that intranasal oxytocin improved social interaction and communication of subjects with ASD.[76,77] These promising results warrant further study to demonstrate the efficacy.

NOVEL TREATMENT

Recent studies on the role of inflammation on ASD have led to the development of novel therapies. In the past, immune-based therapies such as usage of steroid and IVIG have been investigated. Treatment with steroids shows some positive benefits. A single case study reported improvement in language and social skill after daily high-dose steroid.[78] In one series of 32 children, 53% of subjects had improvement in EEG and language skills after prednisone

treatment. However, the benefits reported were all transient. In addition, there is lack of large placebo controlled clinical trial due to side effects such as Cushingoid or long-term steroid effects. In the case of IVIG, there is no good evidence showing efficacy.[79]

Immunomodulatory therapy with less broad side-effects may prove to be more effective regimen. Novel treatment has been delevoped recently. Flavonoid luteolin, which has the effects of anti-inflammation, microglial activation inhibition, and anti-oxidative, was shown to be beneficial in children with ASD.[80] An open-label pilot study showed that dietary supplement containing 2 flavonoids (luteolin and quercetin) improved function in communication, social skill. The side-effect includes transient increased irritability.[80] The side-effect profile appears to be much better than steroid. Luteolin also improved ASD-like behavior in animal models.[81]

Actos is FDA approved for diabetes treatment. It has an anti-inflammatory effect and has been tested in several different neurological diseases. One open-labeled study reports that pioglitazone (Actos) improved irritability, stereotopy, and hyperactivity in those with ASD.[82] In one double-blinded, placebo-controlled trial, combination of the non-steroid anti-inflammatory drug (Celecoxib) with risperidone was shown to have more improvement in repetitive behavior and social withdrawal as compared to risperidone alone treatment.[83]

CONCLUSION

Evidence suggests that neuroinflammation plays an important role in the pathogenesis of ASD from the early stage of the disease until the later phase. Multiple brain regions are involved in the process. Pharmaceutical treatments that reduce neuroinflammation may help to ameliorate the symptoms in ASD.

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CONFLICT OF INTEREST

None.

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