Neuroimmunology of Autism Spectrum Disorder

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ABSTRACT

Autism is a developmental disorder characterized by immunological and neurological abnormalities. The role of cytokines in the pathophysiology of autism has been researched suggesting a relationship with altered blood-brain barrier permeability and subsequent neuroinflammation. Cytokine recruitment to the CNS may result in altered neurotransmitter signaling and the behavioral manifestation of autism symptoms. Other immune mediated events such as changes in the number and activity of natural killer cells, macrophages, immunoglobulins, and glutathione may contribute to altered neuronal signaling and neurotransmitter imbalances. The purpose of this overview is to examine the relationship between immune system and nervous system dysfunction to determine biomarkers for autism spectrum disorder. We will explore the utility of serum cytokines and urinary neurotransmitter analyses as biomarkers for autism.

INTRODUCTION

Autism is a pervasive developmental disorder characterized by impaired development of social interaction and communication, and a markedly restricted repertoire of activities and interests (American Psychiatric Association, 1994). The exact etiology of autism remains largely unknown, however, literature has emerged to suggest genetic, neurological, immunological, and environmental contributions. Immunological and environmental factors, such as diet, infection, and xenobiotics play critical roles in the development of autism. (Ivarsson, Bjerre, Vegfors, and Ahlfors, 1990; Wakefield et al., 1998; Edelson and Cantor, 2002; Fatemi et al., 2002; Kibersti and Roberts, 2002). Abnormalities in enzymatic function (Fatemi et al., 2002a), autoantibodies to brain proteins (Vojdani et al., 2002), and maternal infections during pregnancy (Shi et al., 2003) have been indicated in the autism population. Additionally, pathological alterations in genes involved in the patterning of the central nervous system, biochemical pathways, development of dendrites and synapses, and genes associated with the immune system have been observed in this population (Burber and Warren, 1998; Palmen, Engeland, Hof, and Schmitz, 2004; Polleux and Lauder, 2004; Cohen et al., 2005; Crawley, 2007; Glessner et al., 2009; Wang et al., 2009).

Interestingly, an emerging body of evidence is growing concerning the link between abnormal immune function and neurological dysfunction with autism spectrum disorders. At critical times of infantile development, immune dysregulation may result in the release of immunomodulatory molecules, such as chemokines and cytokines, leading to altered neuronal development and neural function (Cohly & Panja, 2005).

Chemokines and cytokines are proteins that manage immune cell trafficking and cellular arrangement of immune organs and determine appropriate immune responses (Borish & Steinke, 2003). Cytokines can be transported to and/or synthesized in the central nervous system (CNS) thereby establishing communication between peripheral immune cells and CNS neurons (Dunn, 2006). The purpose of this overview is to identify neurological and immunological abnormalities that exist in individuals with autism. Further, it will become critically apparent that neuroimmune biomarker testing for autism can identify these abnormalities and ensure therapeutic effectiveness.

CYTOKINES AND NEUROTRANSMISSION

Cytokines released by immune cells, particularly interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), communicate with the CNS to affect neural activity and modify behaviors, hormone release, and "normal" autonomic function (Dunn, 2006). Cytokines can enter the brain by various mechanisms including active transport or direct entry through a compromised blood-brain barrier. Active transport mechanisms that involve a saturable system have been documented for IL-1 and TNF-α (Dunn, 1992; Gutierrez, Banks, and Kastin, 1993; Gutierrez, Banks, Kastin, 1994). Additionally, Maier and colleagues (1998) found that cytokines may directly enter the central nervous system at circumventricular regions, predominantly the area postrema, where the blood brain barrier is less protective (Pavlov et al., 2003). Other circumventricular regions of potential cytokine entry include the pineal gland, subfornical organ, organum vasculosum of the lamina terminalis, choroid plexus, median eminence, subcommissural organ, and posterior pituitary (Ganong, 2000).

Upon entry into the CNS, cytokines promote regulatory signals in the brain, through augmentation of hypothalamic-pituitary-adrenal (HPA) axis activity and vagal efferents, which can modify peripheral immune status. Enhanced HPA axis release of epinephrine and cortisol can decrease the release of pro-inflammatory cytokines from macrophages in the periphery

(Pavlov et al., 2003). In addition, enhanced vagal efferent activity can trigger the release of acetylcholine from peripheral parasympathetic nerve endings, decreasing the release of pro-inflammatory cytokines (Pavlov et al., 2003). It is therefore evident that the immune system and nervous system communicate to maintain homeostasis, yet under excessive immune challenges alterations in neuronal signaling can develop.

Studies have shown that peripheral activation of cytokines can lead to CNS release of various neurotransmitters. Specifically, IL-1 administration may promote CNS release of norepineprhine, serotonin, dopamine, glutamate, and gamma-amino-butyric-acid (GABA) (Dunn, 1992; Zalcman et al., 1994; Casamenti et al., 1999; Luk et al., 1999; Huang and O'Banion, 1998). With enhanced turnover of these neurotransmitters, significant neurological and behavioral alterations transpire. Research has shown how immune challenges can alter neurotransmission leading to behavioral changes and psychiatric disorders (Kronfol & Remick, 2000). For example, elevated levels of interleukin-6 (IL-6) have been associated with depressive symptoms (Bob et al., 2009).

In Autism, alteration in immune system function may contribute to impaired neurological signaling. A possible mechanism contributing to neuronal dysfunction in the autistic brain is the transport of noxious substances across the blood-brain barrier into the CNS leading to autoimmunity. Studies have shown how cytokines, chemokines, immunoglobulins, and natural killer cells promote the recruitment of noxious chemicals in the brains of autistic individuals, as well as contribute to autoimmunity (Ashwood et al., 2006). Proinflammatory chemokines, such as monocyte chemotactic protein-1 (MCP-1) and thymus activation-regulated chemokine (TARC), along with cytokines, such as TNF-α, were consistently elevated in the brains of individuals with autism (Cohly & Panja, 2005). The transport or synthesis of cytokines in the brain may contribute to neuroinflammation and possible neurotransmitter imbalances (Cohly & Panja, 2005). Furthermore, Ashwood and colleagues (2008) found that reduced levels of the modulatory cytokine, transforming growth factor-β_1 (TGF-β_1), in autistic children contributed to the dysregulation of adaptive behaviors and predisposal for autoimmune responses. Autoimmunity can be detrimental to normal neuronal signaling and result in significant behavioral abnormalities (Ashwood et al., 2006). Vojdani and colleagues (2008) reported decreased natural killer cell activity in autistic children with low intracellular levels of glutathione, IL-2, and IL-15. Decreased natural killer cell activity has been associated with autoimmunity through alteration of cytokine production (Johansson et al, 2005). Lastly, Entrom and colleagues (2009) demonstrated elevated immunoglobulin G_4 (IgG_4) production in children with autism. Elevated IgG antibodies have been identified against brain-specific proteins in the hypothalamus and thalamus of autistic children, again suggesting autoimmunity (Cabanlit et al., 2007).

Although limited studies on autism and autoimmunity exist, it has been hypothesized that the excess transport and synthesis of proinflammatory chemokines, cytokines, and immunoglobulins from the periphery to the CNS contribute to the development of autoimmune responses (Cohly & Panja, 2005). Autoimmunity may lead to dysregulated neuronal signaling causing behavioral manifestation of autism symptoms. Therefore, assessment of immune and nervous system function may provide biochemical targets to treat patients with these behavioral abnormalities.

NERVOUS SYSTEM BIOMARKERS AND AUTISM

Biomarkers are substances used as indicators of a biologic state. Research has revealed the clinical utility of urinary neurotransmitters as practical biomarkers to associate with neurotransmission (Kusaga et al., 2002; Hughes et al., 2004). Urinary neurotransmitter analysis is an innovative, minimally invasive method to assess peripheral neurotransmitter levels, and has a breadth of data to support its usefulness in clinical practice. In the 1950's, research uncovered correlations between urinary catecholamine levels and psychiatric symptoms, such as depression and anxiety (Bergsman, 1959; Carlsson et al., 1959). Recent research has examined the utility of urinary neurotransmitter analysis to categorize subsets of depression and anxiety, and to determine pharmaceutical intervention(s) (Hughes et al., 2004; Otte et al., 2005). Notwithstanding, urinary neurotransmitter analysis can further be used to assess Attention-Deficit-Hyperactivity Disorder (ADHD). Subjects with ADHD tend to have decreased urinary monoamine neurotransmitter levels (specifically, beta-phenylethylamine (PEA)) that can impair mood and attention (Kusaga et al., 2002). What's more, decreased beta-PEA levels may contribute to symptoms of inattentiveness (Berry, 2004).

Overall, urinary neurotransmitter assessment can be a useful tool in any clinical practice, especially those managing psychiatric disorders. Urinary neurotransmitter analysis can identify neurotransmitter abnormalities that may contribute to behavioral changes, and thereby allow more appropriate treatment selection (Kahane, 2009).

In autism, urinary neurotransmitter analysis has been utilized to examine biochemical abnormalities. As such, urinary serotonin has been the primary urinary neurotransmitter evaluated in autistic individuals. Abnormalities in urinary serotonin have

been linked to immunological disturbances. A recent study found consistent elevations in the number of mast cells, along with elevated levels of urinary serotonin, in autistic patients (Castellani et al., 2009). Food, stress, or viruses can stimulate mast cells in the intestines and brains of young children. Localized and systemic immune activation can lead to enhanced cytokine and serotonin release from mast cells and disruption in the lining of the intestines and the blood-brain-barrier causing altered neuronal signaling (Castellani et al., 2009). As mentioned previously, a compromised blood-brain barrier permits noxious substances entry into the brain and contribute to neuroinflammation. CNS neurotransmitter abnormalities may result from neuroinflammation leading to behavioral changes.

As identified in autistic individuals, raised peripheral glutamate levels may also result from a compromised blood-brain barrier (Moreno-Fuenmayor, et al, 1996, Yip, 2007). Elevated plasma glutamate has been attributed to decreased levels of its rate limiting enzyme glutamic acid decarboxylase (GAD) in autistic individuals (Shinohe, 2006, Yip, 2007). Specifically, Fatemi and colleagues (2002a) and Yip and others (2007) reported a reduced number of GAD 65 and 67 proteins in Purkinje cells in autistic cerebella. The decreased GAD may be due to autoantibodies specific for GAD, which has been detected in various neurological disorders (Manto et al., 2007). These autoantibodies attack the body's own cells, tissues, and/or organs, causing inflammation and tissue damage. Because GAD converts glutamate to gamma-immunobutyric acid (GABA), a decrease in this enzyme will cause subsequent increases in glutamate levels (Yip, 2007). Clinically, high glutamate levels can be excitotoxic and may lead to neurodegeneration and cognitive dysfunction (Ha et al., 2009).

Studies have demonstrated that particular biochemical measurements, such as in plasma amino acid levels, are elevated in children with autism when compared to controls. Autistic children demonstrated elevated levels of plasma glutamate and aspartic acid along with taurine, phenylalanine, asparagine, tyrosine, alanine, and lysine (Moreno-Fuenmayor, Borjas, arrieta, Valera, and Socorro-Candanoza, 1996; Aldred, Moore, Fitzgerald, and Waring, 2003). These amino acid alterations may be caused by immune mediated events, vitamin insufficiency, alterations in neurotransmitter transport, or metabolic derangement.

Imaging studies have further revealed abnormalities in autistic individuals, which suggest that abnormal brain growth in many major brain structures such as cerebellum, cerebral cortex, amygdala, hippocampus, corpus collosum, basal ganglia, and brain stem may contribute to behavioral abnormalities in autism (Courchesne et al., 2001; Acosta and Pearl, 2004). Moreover, research shows that reduced cerebellar volume in the autistic brain is due to decreased numbers of Purkinje cells located in the cerebellum. Altered Purkinje cell population can eventually lead to disrupted and weakened motor coordination (Palmen, Engeland, Hof, & Schmitz, 2004). Taken together, abnormal brain growth could be another factor that can contribute to peripheral neurotransmitter imbalances and behavioral manifestation of symptoms.

What's more, abnormal neural development and function may result from cytokine recruitment to the CNS and therefore amino acid and neurotransmitter alterations (Cohly & Panja, 2005). Changes in amino acid levels may lead to elevated or insufficient neurotransmitter activity and thus can interfere with normal cognitive development (Aldred, et al., 2003). During infancy and adolescence, maintenance of optimal neuronal signaling is essential to ensure normal development of attentional processes, memory, and overall cognitive function, lending credence to the importance of early intervention through laboratory analysis of neurotransmitters and cytokines.

CONCLUSION

Immune system and nervous system activity must be viewed and examined as one system functioning in parallel. It is well established that neurological and immunological abnormalities exist in autistic individuals, however, the relationship between neural and immune function has just recently been emphasized. Food, stress, and viruses can activate immune cells in the periphery and result in CNS disruptions. This may lead to inflammation in the brain and eventually to behavior changes (Castellani et al., 2009). Healthcare practitioners should understand and evaluate the status of the nervous system together with the immune system to best optimize therapeutic intervention(s). Through the development of innovative laboratory tests to analyze neurotransmitters and cytokines, comprehensive information can be obtained to determine neurological and immunological abnormalities. These biochemical measures can serve as biomarkers for clinical symptoms, as well as provide significant guidance for therapeutic selection to reestablish physiological homeostasis and to benefit overall health and wellbeing.

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