Review Article

Immunobiology

ROLE OF THE IMMUNE SYSTEM IN THE BIOLOGY OF AUTISM SPECTRUM DISORDERS

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ABSTRACT

Discovery of autoantibodies that target brain proteins in autistic children and their mothers is an interesting finding. The circulating maternal autoantibodies directed towards fetal brain proteins are highly specific for autism. Furthermore, cellular immune system in autistic children suggests that there may be a defect in signaling pathways that are shared by the immune and central nervous systems. Exploration of the role of immune system in neural development is of great interest in recent research, which may have profound implications for diagnosis and treatment of autism. This review is focused on the most recent research concerning the potential role of immune system dysfunction in autism.

KEYWORDS: Autism Spectrum Disorders; Autoantibodies; Cytokines; Immunoglobulin; Neurological comorbidities

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INTRODUCTION

What is ASD

Autism Spectrum Disorders (ASD) are complex heterogeneous neurodevelopmental diseases that are defined by diagnostic criteria such as presence and severity of repetitive/stereotypic behaviors, restricted interests, abnormal social interactions and impaired communication.[1,2] Autistic disorder, which is also called as autism or classical ASD, is a severe form of ASD, where along with ASD, children may appear with conditions, including a milder form of Asperger syndrome, fragile X syndrome (which causes intellectual disability), tuberous sclerosis, epileptic seizures, Tourette syndrome, learning disabilities, and attention deficit disorder.[3] The exact cause of autism and ASD remains largely unknown, but research has shown that several complex combinations of environmental, neurological, immunological, and genetic factors play important roles. Strong genetic links have been shown for cases with tuberous sclerosis, fragile X, neurofibromatosis, and chromosomal abnormalities.[2-5] Impaired social interaction is considered as the hallmark feature of ASD. In some children, the apparent initial signs and symptoms vary widely in the early developmental period; and social deficits and behavioral patterns might not be recognized as symptoms of ASD or they may be masked by learning strategies in later life. Therefore, ASD remains undiagnosed for a few years until a child is unable to meet social, educational, occupational, or other important life stage demands.[6] Hence persons with ASD have varied functional limitations which might develop over time.

Global prevalence

Lack of awareness about mental health, poor medical infrastructure and social stigma contribute to the poor understanding of autism prevalence in many countries. This makes it difficult to pin down genetic, environmental and cultural factors that may affect prevalence. Elsabbagh *et al*., (2010), reviewed epidemiological surveys of autistic disorder and pervasive developmental disorders worldwide and estimated a prevalence of ASD to a median of 62 cases per 10,000 people.[7] The number of children known to have autism has increased dramatically since the 1980s. There is a lack of evidence from less developed countries;[7] this may be partly due to changes in the diagnostic practices. It is unclear whether prevalence has actually increased.[8] In USA, about 1 in 68 children has ASD and it is about 4.5 times more common in boys (1 in 42) than among girls (1 in 189).[6] It can affect children of all racial, ethnic, and social groups wherever they live.[7]

Diagnosis

Currently, diagnoses of ASD are based on phenotypic characteristics and there are no trusted laboratory tests available that can assist clinicians. Several tools have been developed for the diagnosis of autism; however, the need for clinically useful and reliable biomarkers remains strong. Genomic studies on autism have revealed numerous genetic copy number variants, short nucleotide polymorphisms and common risk variants which may enhance the susceptibility for ASD.[4,9] Despite the recent advances in genetic studies, an explanation for the disorder remains elusive and reliable genetic markers have not yet been identified.[10] However, a number of non-genetic potential markers have been identified, and the immunological connections with ASD are frequently described. Therefore this review aims at providing an overview of the various immune aberrations described among individuals with ASD and their family members.

IMMUNE SYSTEM ABERRATIONS AND AUTISM

Immunological variations, such as altered levels of immunoglobulins, cytokines, inflammation, and cellular activation have been noted in individuals with autism.

Immunoglobulin levels

Immunoglobulins (Igs) are proteins produced by B cells that specifically target entities for destruction and removal. There are several classes of Igs, each with a specific role in immunological processes.[11] Abnormal concentrations of plasma IgG and IgM have been described in some ASD children.[5,12] An association of the levels of Igs with behavior severity showed that individuals with autism having extremely severe behavioral symptom scores had the lowest IgG and IgM levels.[12] Further characterization of IgG subclasses demonstrated that young children with autism have significantly higher levels of IgG4 compared with age matched typically-developing children.[13] Croonenberghs *et al*., (2002), observed increased levels of IgG2 and IgG4 in serum.[14] Yet another study showed distorted IgG1 and IgG4 as well as IgM and IgG levels in ASD.[15] Although the relationship between reduced total Igs and behavior is unclear, it may be possible that defects in shared signaling pathways may lead to both, i.e., altered neurodevelopment and immune function; studies are currently underway to examine this hypothesis. Alterations in various immune cells including natural killer cells and macrophages have been noted in individuals with autism and reviewed by Goines *et al*., (2010).[16] The production of IgG4 antibodies appears to be driven in part by T helper 2 (Th2) cytokines that mediate allergic responses and IgE production.[17] Furthermore, potential gene-environment interactions, in which chronic antigen exposure of individuals who have a genetic predisposition towards immune dysregulation could thus, lead to increased IgG4 concentrations. The link between such immune dysregulation and changes in neurodevelopment and, subsequently behavior, are still unknown.[13]

Altered cytokine profiles and autism

Cytokines are the key immune mediators that facilitate immunological communication.[18] They are proteins of 8–25 kDa size and include interleukins (IL), chemokines, interferons (IFN), tumor necrosis factors (TNFs), and growth factors. Cytokines are highly pleiotropic, and can act in either autocrine, paracrine, and/or in endocrine fashions. Primary source of cytokines are various immune cells such as dendritic cells, macrophages, neutrophils, T cells, B cells, and additional cell types which include neural cells that produce and respond to them. Cytokines act mainly as mediators of immunological activity but they are also involved in the

regulation of neuronal cell migration, proliferation, differentiation, apoptosis and in neuronal communication with microglia.[19,20] Therefore, the altered activity of cytokines may lead to a variety of neurological implications in ASD. A recent report suggests that autistic subjects show a varied level of inflammatory cytokines in the blood mononuclear cells, serum, plasma, brain tissue, and cerebrospinal fluid in comparison with normal subjects.[21] Chronic inflammation and activation of microglial cells has been observed in brain autopsy of people with ASD.[22,23] This might be due to deregulated cytokin levels that impair immune capacity in the central nervous system (CNS) or stimulated production and activation of microglia in the brain.[22,24] Activated microglia can induce the production of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α, which are typically intended to prevent further damage to brain tissue.[20,21] Increased expression of IFN-γ and IL-12 can positively amplify the inflammation, whereas increased production of transforming growth factor-β (TGF-β) can negatively regulate inflammation.[25] Since cytokines are permeable in to blood brain barrier and give rise to oxidative stress, acutely stressful situations, elevated homocysteine, diabetes, and hyperglycemia[23] which can further complicate ASD. IL-1, IL-2, TGF-β, and granulocyte-macrophage colony-stimulating factor (GM-CSF) expression found are inconclusive as different results have been found from different tissues.[20] However, the probable explanation could be that upon crossing blood brain barrier, IL-1 stimulates its own expression in the hypothalamus, which leads to neuroendocrine changes associated with fever and sickness.[26,27] In addition, the receptors of IL-1 are structurally related to toll-like receptors (TLRs), and signaling may be achieved through Nuclear factor-κB (NF-κB) and Mitogen-activated protein kinases (MAPKs) signaling cascades[28] which are the determinant of innate immune and inflammatory responses. IL-4 also has an important role in producing the regulatory cytokine IL-10, which helps maintain a balance between the cytokines produced by T Helper (Th) cells 1 and 2. An imbalance in Th1/Th2 could also play a role in the pathogenesis of autism.[5] Although children with ASD showed increased activation of both Th2 and Th1, with a predominance of Th2, there was no significant increase in the regulatory cytokine IL-10.[20,29] Recently Ross et al., (2013), showed that the individuals with 22q11DS, a syndrome with increased risk for developing autism, had higher levels of IL-10 and were inversely related to autism-related behaviors.[30] Additionally, the ratio of pro- to anti-inflammatory cytokines, as determined by IL-6 : IL-10 ratio, was associated with social behavior deficits.[30] Elevated plasma cytokines seemed to correlate with regressive onset and severity of autistic and behavioral symptoms.[31] Alteration of pro-inflammatory cytokines, complement proteins, chemokines, adhesion molecules, and growth factors are associated with ASD.[24,29,31] In addition, elevated levels of pro-inflammatory and decreased levels of regulatory cytokines in the CSF and peripheral blood are associated with ASD.[5,24,31-34] More specifically, altered TGF-β, CCL2, CCL5, IgM and IgG classes of circulating Ig levels are linked with a worsening of behavioral scores.[35] IL-10, an anti-inflammatory cytokine that contributes to intestinal homeostasis, An elevated level

of neopterin, which is synthesized by macrophages upon stimulation with γ-IFN, is associated with increased production of reactive oxygen species and can be considered as a measurement of the oxidative stress elicited by the immune system.[36] Neopterin levels were found significantly higher in serum samples from individuals with ASD in comparison with the controls, which may imitate increased cell-mediated immune activation and IFN-γ production.[32] In addition, neopterin and biopterin were shown to be increased in urine samples of ASD children.[37] In vitro experiments demonstrated the decreased proportions of IFN-γ and IL-2 synthesizing CD4+ (Th1) and CD8+ (TC1) T cells in autistic children as compared to controls. However, IL-4 synthesizing CD4+ (Th-2) and CD8 (TC2) T cells were significantly higher in autism.[38] Al-Ayadhi et al., (2012), showed an increase in a calcium binding protein S100B production by astrocytes as a result of neural damage in ASD and which was found to be associated to autistic severity.[39] Interestingly, increased TNF-α was found in primary sibling family members of patients with ASD, demonstrating similar immune deregulation and genetic susceptibility in the patients studied.[5] Cytokines and other products of immune activation can affect many behaviors including mood, sleep, appetite and nutritional uptake, exploratory behavior, and social interactions. For example, administration of therapeutic doses of systemic cytokine, IFN-α, IL-2, and TNF-α had effects, including mood depression, sleep disorder, impaired cognitive function, decreased exploratory behavior, and changes in motivation.[40] Systemic administration of cytokines can also induce increased noradrenergic, dopaminergic, and serotonergic metabolism in the hypothalamus, hippocampus, and nucleus accumbens and modulate synaptic plasticity and thereby alter memory and learning.[41] Overall, these studies involving administration of cytokines indicate that a more complex pattern of cytokine production occurs in autism and differences between studies may be indicative of a possible patient selection bias and that particular cytokine profiles may potentially reflect different autism behavioral phenotypes.

MATERNAL ANTIBODIES AND AUTSIM

The maternal immune system has been associated with the pathogenesis of some forms of ASD. IgG can cross the placenta during pregnancy which probably affect brain development leading to a type of ASD. Recent studies have revealed that the presence of specific maternal IgG autoantibodies which react with the fetal brain proteins of children who exhibit ASD.[42,43] Case reports and several epidemiological studies suggest that maternal prenatal exposure to viral or bacterial infections could be a possible reason for initiation of ASD in some children.[44,45] Maternal antibodies cross the underdeveloped blood brain barrier of the fetus[46] leading to impaired fetal neurodevelopment and long-term neurodegeneration, neurobehavioral, and cognitive difficulties.[47] A maternal infection or immune response includes cytokines, which also affect aspects of fetal neurogenesis, neuronal migration, synaptic plasticity, and stem cell fate.[48] It was observed that elevated levels of serum IFN-γ, IL-4, and IL-5 were more common in

women who gave birth to a child subsequently diagnosed with ASD.[16] Exposure of fetal IL-6 in late pregnancy can lead to abnormalities of hippocampal structure and morphology, and decreased learning during adulthood.[47] Some of the antibodies cross the developing blood brain barrier of fetus that recognize and attack the fetal brain.[49] Antibodies directed against fetal brain protein in ASD can be an inappropriate approach to unfamiliar peers.[42] Braunschweig *et al*., (2013), developed a panel of clinically significant maternal autoantibody-related autoantibody biomarkers with over 99% specificity for autism risk.[50] This panel has suggested that an early diagnosis of maternal autoantibody-related autism allow for interventions that limit fetal exposure to these antibodies and allow early behavioral therapies.[23]

NEUROLOGICAL IRREGULARITY AND AUTISM

The interaction of immune system and nervous system is extensively complexes with ample communication occurring between them in health and disease. Therefore immune dysfunction is frequently noted in neurological disorders. Neurobiological studies also highlighted atypical neural development, synapse plasticity, structural brain abnormalities, cognition and behavior. Additionally, abnormalities in the cellular immune response have also been reported in children with autism; in particular, reduced cytotoxic activity and elevated pro-inflammatory cytokine levels produced by peripheral blood mononuclear cells, such as TNF-α and IL1β, have been shown to disrupt neurodevelopment.[13,33] Cytokines and their products in the immune system, can modulate brain function, affecting cognitive and emotional processing, and have assorted effects on neuronal tissue, such as the modulation of systemic and CNS responses to infection, injury, and inflammation.[40] Neurological comorbidities in ASD are not only common, but they are also associated with more clinical severity. Abnormalities in normal neurodevelopment that are suggestive of ASD have been extensively studied.[51] Neurodevelopment occurs in multiple, critical time points, including the phases of proliferation, migration, differentiation, synaptogenesis, gliogenesis, myelination and apoptosis of neurons. Overlap of these phases are considerable which extend from the embryonic stage up until adolescence. Magnetic resonance imaging (MRI) studies of postmortem brains of ASD patients showed neurological and structural abnormalities in the cerebellum, hippocampus, amygdala and insular cortex.[2] Neurological comorbidities such as motor dysfunction, sleep impairment and epilepsy are prevalent in ASD and are associated with a more severe phenotype.[51]A meta-analysis showed that in subjects with ASD, at birth, brain volume is smaller by an average of 13%, whereas at 1 year of age, brain volume was larger by an average of 10% in comparison with controls, and 2% larger in adolescence.[53,54] An increase in gray matter with a reduced unit density,[53] increase in CSF volume with enlarged ventricles[54] and decrease in CSF volume with mini-columnar[55] have also been reported. It has been proposed that such structural variation may affect adaptation and hence learning, and may account for the

heterogeneity and wide-ranging functional deficits seen in ASD.[55] Neuroglial activation and neuro-inflammation have been seen in the brain of patients with autism involving excess microglial activation and increased pro-inflammatory cytokine profiles.[24,30,31,56] Other immune abnormalities such as lower TGF beta 1 levels were associated with lower social adaptive behaviors and worse behavioral outcomes such as irritability, lethargy, stereotypy and hyperactivity.[5]

AUTOIMMUNITY AND AUTISM

Children with ASD were found to have a family history of autoimmune disorders, including asthma, multiple sclerosis (MS), rheumatoid arthritis, Type 2 diabetes mellitus and celiac disease. Immune responses associated with allergy may contribute to the pathogenesis of ASD, as allergy may induce the production of brain-specific autoantibodies secondary to the allergen exposure.[45,57] Increasing evidence of autoimmune phenomenon is proposed as a distinct, objectively defined etiologic subtype of ASDs.[58] Immune responses associated with allergy may have a role in the pathogenesis of language and social developmental abnormalities in a subset of children with these disorders.[13, 59,60]Autoimmunity was first linked to autism in a study of a child with autism, who had an expansive family history of autoimmune diseases.[61] This study proposed that an inherited risk of autoimmunity could increase the risk of developing autism. Sweeten *et al*., (2003), showed that first- and second-degree relatives of children with an ASD have a higher number of autoimmune disorders than those of healthy children.[32] In a recent post-mortem study of 13 males with autism, microglia appeared markedly activated in 5 cases, including 2 (of 3) under the age of 6 years, and marginally activated in an additional 4 cases, suggesting ongoing inflammatory processes in brain.[62] These observations are also supported by animal experiments. Exposure of pregnant rhesus monkeys to human IgG collected either from mothers with ASD children, or from mothers of normal children, consistently demonstrated increased whole-body stereotypes and hyperactivity in comparison with the controls.[63] These findings suggest that environmental manipulations can trigger some ASD-like behaviors in presence of anti ganglioside M1 antibodies, antineuronal antibodies, and serum anti-nuclear antibodies which are associated with the severity of autism.[39] A number of other autoantibodies such as anti-neuron-axon filament protein (anti-NAFP) and anti-glial fibrillary acidic protein (anti-GFAP), antibodies to brain endothelial cells and nuclei, antibodies against myelin basic protein,[64] and anti-myelin associated glycoprotein, as an index for autoimmunity in the brain[64] are also suggestive of a pathological role in autism. Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin, present in low levels, but with an impact on brain development and function, may be involved in the pathophysiology of ASD.[65]

CONCLUSION

Decades of research links immune dysfunction to autism. The immune and nervous system maintain extensive cross-talk including shared signaling and developmental pathways. More evidence is required to establish a relationship between reduced total Igs and behavior, alteration in complex pattern of cytokine activity, neurological implications, and maternal autoantibodies and their relation to autism. Thus, understanding of immunological changes in autism may probably lead to finding key biological markers for ASD that will have implications in early diagnosis and therapeutic interventions.

CONFLICT OF INTEREST
None.

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