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Original article

Autoantibodies against neuronal progenitors in sera from children with autism

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Abstract

The pathological role of autoantibodies in development of CNS disorders is a new idea with growing interest among neuroscientists. The involvement of autoimmune response in the pathogenesis of autism spectrum disorders (ASD) has been suggested by the presence of multiple brain-specific autoantibodies in children with ASD and in their mothers. The possibility of the effect of autoimmunity on neurogenesis and postnatal brain plasticity has not been determined. The presence of autoantibodies against human neuronal progenitor cells (NPCs) stimulated for neuronal differentiation in culture was tested in sera from children with autism ($n = 20$) and age-matched controls ($n = 18$) by immunoblotting and immunocytochemistry. Immunoreactivity against multiple NPCs proteins of molecular sizes of approximately 55 kDa, 105 kDa, 150 kDa, and 210 kDa in sera from individuals with autism had a higher incidence and was stronger than in control sera which immunoreacted mainly with a 150 kDa protein. The sera from children with autism immunoreacted the strongest with NPCs expressing neuronal markers Tuj1 and doublecortin, but not astrocyte marker GFAP. The epitopes recognized by antibodies from sera were not human-specific because they detected also NPCs *in situ* in murine hippocampus. The autoimmune reactions against NPCs suggest an impaired tolerance to neural antigens in autism. These autoantibodies may be symptomatic for autism and furthermore, their presence suggests that autoimmunity may affect postnatal neuronal plasticity particularly after impairment of blood–brain barrier. Future studies will determine the diagnostic value of the presence of autoantibodies in autism and the therapeutic value of prevention of autoimmunity in autism.
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Keywords: Autism; Autoantibodies; Neurogenesis; Cell culture; Neuronal progenitor cells

1. Introduction

The evidence for involvement of autoantibodies against neuronal surface and synapse antigens in pathogenesis of central nervous system (CNS) disorders has recently emerged in neuroscience society [1–3]. Numerous reports suggest that altered interactions of the immune and central nervous systems (CNS) may contribute to the development of autism spectrum disorders (ASDs)—the pediatric neuropsychiatric syndrome of high incidence [4,5]. Brain-specific autoantibodies have been detected in children with ASD and their mothers at frequencies significantly higher than in control children, which suggest a possible effect of autoimmune

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processes on developing brain [6–9]. In families with a child with autism, the incidence of autoimmunity is significantly higher than in the general population, suggesting that a specific genetic background may facilitate the development of autism [10]. Most children with autism have increased levels of anti-ganglioside M1 antibodies—an indicator of autoimmunity to the CNS—and the levels positively correlate with scores on the Childhood Autism Rating Scale [8]. Moreover, children with autism show increased serum levels of the peptide neurotensin (NT), which induces extracellular release of mitochondrial DNA, which could activate an autoimmune response [11].

The findings of a positive correlation between viral antibodies and autoantibodies, and elevated levels of proinflammatory cytokines and acute-phase reactants as well as a positive response to immunotherapy have suggested a possible role of viral infection in the pathogenesis of ASD by triggering an autoimmune response against brain targets [12]. The effect of autoantibodies on early brain development results in abnormal behavior, as has been demonstrated in rhesus monkeys and in rodents after *in utero* exposure to IgG from mothers of children with autism [13–15]. However, the specific target for autoantibodies that may identify the pathogenic mechanisms involved in the neurobiology of autism has not been identified. Autoantibodies from sera of mothers of children with autism recognize multiple targets in human fetal brain, as demonstrated by immunoblotting [6,16].

Maternal autoantibodies remain in the baby's circulation for several months until production of own antibodies and memory cells begins. Hence, the presence of autoantibodies against neuronal targets in children with autism over 1-year-old indicates alterations of the adaptive immunity in children with autism that result in autoantibodies' production. This may have lifelong consequences on neuro-immunological interactions [9,17–20].

We addressed the possibility that autoimmune response in autism may have effects on neurogenesis, i.e., development of new neurons from neuronal progenitor cells (NPCs), a process known to be ongoing through the postnatal life and relevant for memory formation, spatial learning, pattern separation, fear conditioning, and anxiety in mammals [21,22]. To test this, we evaluated sera from children with autism for the presence of autoantibodies directed against human NPCs stimulated to neuronal differentiation in culture. The results indicated the presence of autoantibodies in sera from children with autism directed against multiple proteins expressed by differentiating NPCs. The presence of autoantibodies against NPCs may contribute to the pathogenesis of autism.

2. Materials and methods

2.1. Serum sample collection

Studies with the use of human samples were approved by our institute's Institutional Review Board. Blood samples were collected among the patients of the IBR clinic originated from families living in the NYC area. Diagnosis of autism was performed on the basis of criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV), using information gathered from the Autism Diagnostic Observation Schedule-Generic (ADOS-G), the Autism Diagnostic Interview-Revised (ADI-R), and the Pervasive Developmental Disorders Behavior Inventory [23]. The Vineland Adaptive Behavior Scale was also used to characterize the children. For the studies were selected blood samples from children without history of seizures or gastrointestinal disturbances; and without fragile X syndrome or Rett syndrome. They were not treated with antidepressants, neuroleptics, seizure medications, or stimulants and they were healthy during blood collections. Collected sera were stored at −20 °C until the experiment. The presence of antibodies against NPCs was tested in sera collected from two heterozygous twin pairs: control and affected brothers (Family A) and an affected brother and sister (Family B), and in sera from unrelated children with autism ($n = 20$) and typically developing age-matched control children ($n = 18$) (Table 1).

2.2. Cell culture

The ability of sera to react with NPCs was tested using human multipotential NPCs (Lonza, Allendale, NJ). These are immature cells which express markers specific for both neuronal (β-tubulin III) and glial (glial fibrillary acidic protein GFAP) lineages and can be stimulated in culture to differentiate into neurons and glia [24]. The neuronal differentiation of NPCs in culture models neurogenesis during prenatal brain development and in adult brain in neurogenic areas [25]. NPCs were expanded as free-floating neurospheres according to cell supplier protocol—in neural progenitor basal medium with human recombinant basic fibroblast growth factor, human recombinant epidermal growth factor, neural survival factor-1, and gentamicin/amphotericin-B (Lonza) for 3 weeks. Half of the medium was changed every 2 days, and neurospheres were passaged every 5–7 days by mechanical dispersion to maintain cell-to-cell contact. Neuronal differentiation of NPCs was induced by planting the neurospheres in poly-D-lysine/laminin-pre-coated plates (BD Bioscience, Bedford, MA) and cultured in differentiating medium (Lonza) without