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Research Report

Altered development of neuronal progenitor cells after stimulation with autistic blood sera

Bozena Mazur-Kolecka[a,*], *Ira L. Cohen*[b], *Edmund C. Jenkins*[c], *Wojciech Kaczmarski*[a], *Michael Flory*[d], *Janusz Frackowiak*[a]

[a]*Department of Developmental Neurobiology, New York State Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Rd, Staten Island, NY 10314, USA*
[b]*Behavioral Assessment and Research Laboratory, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA*
[c]*Department of Cytogenetics, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA*
[d]*Research Design and Analysis Service, New York State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314, USA*

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ABSTRACT

Changes of brain structure and functions in people with autism may result from altered neuronal development, however, no adequate cellular or animal models are available to study neurogenesis in autism. Neuronal development can be modeled in culture of neuronal progenitor cells (NPCs) stimulated with serum to differentiate into neurons. Because sera from people with autism and age-matched controls contain different levels of numerous biologically active factors, we hypothesized that development of human NPCs induced to differentiate into neurons with sera from children with autism reflects the altered early neuronal development that leads to autism. The control and autistic sera were collected from siblings aged below 6 years that lived in the same environment. The effect of sera on differentiation of NPC neurospheres into neuronal colonies was tested in 72-h-long cultures by morphometry, immunocytochemistry and immunoblotting. We found that sera from children with autism significantly reduced NPCs' proliferation, but stimulated cell migration, development of small neurons with processes, length of processes and synaptogenesis. These results suggest that development of network of processes and synaptogenesis – the specific events in the brain during postnatal ontogenesis – are altered in autism. Further studies in this cell culture model may explain some of the cellular alterations described in autistic patients.

1. Introduction

Unknown developmental defects of brain growth, structure, and function cause autism spectrum disorder (ASD), defined clinically as an alteration of cognitive, linguistic, social, and emotional functions (Acosta and Pearl, 2003). Among the neurodevelopmental alterations that could underlie the pathophysiology of autism, the most frequently observed are increased volumes of whole brain, parieto-temporal lobe, and cerebellar hemisphere, as well as abnormal sizes of amygdala,

* *Corresponding author*. Fax: +1 718 494 4856.
E-mail address: Bozena.Mazur-Kolecka@omr.state.ny.us (B. Mazur-Kolecka).

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hippocampus, and corpus callosum (Brambilla et al., 2003; Courchesne and Pierce, 2005b). Moreover, altered cyto-architectural organization of cerebral cortex minicolumns (Casanova et al., 2002), and changes in cell sizes and cell density in the limbic system and cerebellum have been reported (Acosta and Pearl, 2003). These neuropathological abnormalities can be caused by errors that occurred early in ontogeny, as suggested by the increased frequency of malformations associated with ASD that are caused by developmental insults as early as the 4th to 6th week of embryogenesis (Miller et al., 2005).

Pre-programmed neurogenesis, i.e., neuronal proliferation, migration, differentiation, growth and circuit organization, may be strongly affected by factors present in the cellular microenvironment, such as neurotrophins, neuropeptides, regulatory proteins and neurotransmitters, the levels of which are altered in the brain, cerebrospinal fluid, and blood of individuals with autism (Acosta and Pearl, 2003). Neonatal blood spots of individuals who later developed autism and/or mental retardation, contained elevated levels of brain-derived neutrophic factor, neurotrophin 4/5, vasoinhibitory peptide, and the calcitonin gene-related peptide (Nelson et al., 2001; Miyzaki et al., 2004; Tsai, 2005). Blood of some autistic patients contains altered levels of serotonin, a monoamine neurotransmitter that regulates neurogenesis, neuronal differentiation, neuropil formation, axon myelination, and synaptogenesis (Anderson et al., 1990; Chugani et al., 1999; Chugani, 2002; Whitaker-Azmitia, 2005). Altered levels of other neurotransmitter that participate in neuronal integration and interneuron migration such as GABA have been detected in autistic youngsters (Dhossche et al., 2002). Plasma from autistic patients contained increased amounts of total nitrite (Zoroglu et al., 2003), a metabolite of nitric oxide, a diffusible intercellular messenger expressed by nitrergic neurons participating in sensory motor function (Moreno-Lopez et al., 1996), and synaptic formation and remodeling (Holscher, 1997). Nitric oxide, which has been suggested to be a physiological inhibitor of neurogenesis (Moreno-Lopez et al., 2004), is increased in red blood cells in autistic patients (Sogut et al., 2003). These examples of alterations in body fluid concentrations of some factors confirm that the internal environment in people with autism is disrupted. Most authors conclude that altered levels of these factors in the periphery may reflect changes in brain development that underlie autistic pathology, although the mechanisms of such periphery–brain interactions during early development and during postnatal life are unclear. Not fully functional blood–brain barrier during early brain development may be responsible for disruption of serotonin terminals in the brain through a negative feedback function due to high levels of serotonin in the blood (Whitaker-Azmitia, 2005). In rats exposed prenatally to thalidomide or valproic acid, regarded as an animal model of autism, increased concentrations of serotonin were detected simultaneously in both the brain and blood on postnatal day 35 (Narita et al., 2002), suggesting that autism-inducing factors have the same continuous effect on the monoamine system in the brain and the periphery during development and in postnatal life. Hence, the autism-inducing factors may influence development of neurons and neuronal network (neurogenesis) that takes place during early brain development, postnataly and also during adult life (Johansson et al., 1999; Nunes et al., 2003; De Graaf-Peters and Hadders-Algra, 2006; Quinones-Hinojosa et al., 2006; Taupin, 2006).

The critical steps of early neurogenesis can be studied in cultures of neuronal progenitor cells (NPCs) isolated from human fetal tissues or from mature brain. These cells maintain the ability to differentiate into distinct brain cell lineages, i.e., neurons and glia (Gage et al., 1995; Carpenter et al., 1999; Temple and Alvarez-Buylla, 1999; Gage, 2000). Self-renewal and differentiation of NPCs into specific neuronal phenotypes can be stimulated in culture with specific growth factors, cytokines, and neurotransmitters (Weiss et al., 1996; Cameron et al., 1998; Sogut et al., 2003; Temple and Alvarez-Buylla, 1999; Gage, 2000; Ostenfeld and Svendsen, 2003). Development and differentiation of NPCs in culture can be triggered by blood serum that contains a mixture of numerous regulatory factors (Johe et al., 1996). Because the local environment is the predominant determinant of NPC differentiation (Cao et al., 2002a), we hypothesize that sera from people with autism – the sera that show altered levels of regulatory proteins – will differently affect the development of human NPCs (HNPCs) in culture. The alterations of neurogenesis in culture may reflect alterations of neuronal development during postnatal life in people with autism. To test this hypothesis, the development of neurospheres of HNPCs into colonies of neurons was studied after stimulation with sera from individuals with autism and age-matched controls.

Our results suggest that the altered body environment in autism could influence migration of NPCs, development of network of processes and synaptogenesis – the specific events in ontogenesis that characterize postnatal brain development. Our new cell culture model may be the tool to identify the specific cellular mechanisms involved in the altered neurogenesis that may lead to the structural and functional changes that are observed in autism.

2. Results

2.1. *Electrophoretic patterns of serum protein*

Capillary zone electrophoresis was used to semiquantitate the total amount of serum proteins and five main protein fractions, i.e., gamma, beta, alpha 1 and alpha 2 globulins, and albumins. Electropherograms from control and autism cases revealed similar patterns without individual peaks within globulin fractions (representative electropherograms shown in Fig. 1A). Total serum protein levels and protein levels in five main fractions in control and autistic age-matched cases did not differ significantly (Fig. 1B).

2.2. *Characterization of cells grown from neurospheres of HNPC*

Neurospheres of HNPCs cultured for 72 h in control conditions (NBM enriched with 1% human serum from healthy donors) formed colonies of cells that all were immunoreactive for