

Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism

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ABSTRACT Research into the metabolic phenotype of autism has been relatively unexplored despite the fact that metabolic abnormalities have been implicated in the pathophysiology of several other neurobehavioral disorders. Plasma biomarkers of oxidative stress have been reported in autistic children; however, intracellular redox status has not yet been evaluated. Lymphoblastoid cells (LCLs) derived from autistic children and unaffected controls were used to assess relative concentrations of reduced glutathione (GSH) and oxidized disulfide glutathione (GSSG) in cell extracts and isolated mitochondria as a measure of intracellular redox capacity. The results indicated that the GSH/GSSG redox ratio was decreased and percentage oxidized glutathione increased in both cytosol and mitochondria in the autism LCLs. Exposure to oxidative stress *via* the sulfhydryl reagent thimerosal resulted in a greater decrease in the GSH/GSSG ratio and increase in free radical generation in autism compared to control cells. Acute exposure to physiological levels of nitric oxide decreased mitochondrial membrane potential to a greater extent in the autism LCLs, although GSH/GSSG and ATP concentrations were similarly decreased in both cell lines. These results suggest that the autism LCLs exhibit a reduced glutathione reserve capacity in both cytosol and mitochondria that may compromise antioxidant defense and detoxification capacity under prooxidant conditions.—James, S. J., Rose, S., Melnyk, S., Jernigan, S., Blossom, S., Pavliv, O., Gaylor, D. W. Cellular and mitochondrial glutathione redox imbalance in lymphoblastoid cells derived from children with autism. *FASEB J.* 23, 2374–2383 (2009)

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AUTISM IS A BEHAVIORALLY DEFINED neurodevelopmental disorder characterized by impairments in social interaction and communication skills and by hyperfocused interests and compulsive behaviors. Autism is usually diagnosed before 4 yr of age and is estimated to affect 1 in 150 children in the United States, with a 4:1 male to female gender bias (1). Although multiple interacting genetic and environmental factors are

thought to influence individual vulnerability to autism, none have been reproducibly identified in more than a fraction of cases. In addition to complex gene-environment interactions, the heterogeneous presentation of behavioral symptoms within the spectrum of autistic disorders suggests a variable and multifactorial pathogenesis.

Several lines of evidence suggest that underlying oxidative stress and glutathione depletion contribute to pathophysiology of several neurobehavioral disorders, including schizophrenia (2, 3), bipolar disorder (4, 5), Parkinson's disease (6, 7), Alzheimer's disease (8, 9), and autism. Children with autism have been shown to exhibit evidence of lipid peroxidation (10, 11), reduced antioxidant activity (10, 12, 13), elevated nitric oxide levels (14, 15), and accumulation of advanced glycation end products (AGEs) and the proinflammatory AGE receptor ligand S100A9 (16). The presence of redox imbalance and chronic oxidative stress in autism is further supported by evidence of microglial inflammation (17) and decreased glutathione-mediated redox status (18, 19). Although provocative, it is not clear whether these measures of oxidative stress are present during early development and contribute to pathogenesis of autism, or whether they are a secondary manifestation of the disorder.

Oxidative stress is traditionally defined as an imbalance between oxidant generation and antioxidant defense mechanisms that leads to macromolecular damage and dysfunction. More recently, the definition has expanded to include more subtle perturbations in redox signaling mechanisms that control and regulate a wide variety of cellular functions, including enzyme activation/inhibition (20, 21), membrane signal transduction (22, 23), transcription factor binding/gene expression (24, 25), proliferation/apoptosis (26–28), and precursor cell ontogeny (29, 30). The ratio of reduced glutathione (GSH) to the oxidized disulfide form of glutathione (GSSG) is considered a reproduc-

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