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Pathophysiology 13 (2006) 171–181

ISP PATHOPHYSIOLOGY

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Review

Oxidative stress in autism

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Abstract

Autism is a severe developmental disorder with poorly understood etiology. Oxidative stress in autism has been studied at the membrane level and also by measuring products of lipid peroxidation, detoxifying agents (such as glutathione), and antioxidants involved in the defense system against reactive oxygen species (ROS). Lipid peroxidation markers are elevated in autism, indicating that oxidative stress is increased in this disease. Levels of major antioxidant serum proteins, namely transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein), are decreased in children with autism. There is a positive correlation between reduced levels of these proteins and loss of previously acquired language skills in children with autism. The alterations in ceruloplasmin and transferrin levels may lead to abnormal iron and copper metabolism in autism. The membrane phospholipids, the prime target of ROS, are also altered in autism. The levels of phosphatidylethanolamine (PE) are decreased, and phosphatidylserine (PS) levels are increased in the erythrocyte membrane of children with autism as compared to their unaffected siblings. Several studies have suggested alterations in the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase in autism. Additionally, altered glutathione levels and homocysteine/methionine metabolism, increased inflammation, excitotoxicity, as well as mitochondrial and immune dysfunction have been suggested in autism. Furthermore, environmental and genetic factors may increase vulnerability to oxidative stress in autism. Taken together, these studies suggest increased oxidative stress in autism that may contribute to the development of this disease. A mechanism linking oxidative stress with membrane lipid abnormalities, inflammation, aberrant immune response, impaired energy metabolism and excitotoxicity, leading to clinical symptoms and pathogenesis of autism is proposed.

Keywords: Autism; Pervasive developmental disorders; Oxidative stress; Lipid peroxidation; Immune response; Inflammation; Mitochondrial dysfunction; Excitotoxicity

Contents

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doi:10.1016/j.pathophys.2006.05.007

Autism is a severe neurodevelopmental disorder with onset prior to 3 years of age [1]. It is a heterogeneous disorder, both etiologically and phenotypically. Autism is a behaviorally defined disorder and is classified under the pervasive developmental disorders (PDDs). PDDs are a group of disorders that involve a combination of impairments in communication, reciprocal social interactions, and stereotyped patterns of interest/behavior. PDDs include autism, Asperger's syndrome (a similar condition that is not associated with language delay or general intellectual impairments), Rett's disorder, Childhood disintegrative disorder, and PDD—not otherwise specified.

While the cause of autism remains elusive, autism is considered a mutifactorial disorder that is influenced by genetic, environmental, and immunological factors as well as increased vulnerability to oxidative stress. No single gene has been found to be associated with autism, and involvement of multiple genes has been postulated [2–5]. Environmental factors, such as mercury, lead, measles, rubella virus, retinoic acid, maternal thalidomide, valproic acid and alcohol use during pregnancy have been suggested to be involved in the etiology of autism [6–10]. In addition to behavior impairments, gastrointestinal disturbances [11–15] and epilepsy [16] have been described in some patients with autism. Immune [3,17–19], autoimmune [20–22], and infectious factors [8,9,23–27] have also been suggested to play role in the etiology of autism. Increasing evidence suggests a role of oxidative stress in the development and clinical manifestation of autism [28,29]. In fact, oxidative stress has also been implicated in the pathogenesis of other neuropsychiatric diseases, including schizophrenia [30–32] and major depressive disorder [33], anxiety disorders such as panic disorder [34], and obsessive-compulsive disorder [35]. It is suggested that autism may result from an interaction between genetic, environmental, and immunological factors, with oxidative stress as a mechanism linking these risk factors.

1. Oxidative stress

Under normal conditions**,** a dynamic equilibrium exists between the production of reactive oxygen species (ROS) and the antioxidant capacity of the cell [36,37]. ROS includes superoxide ($O_2^{\bullet -}$), hydroxyl, peroxyl, alkoxy, and nitric oxide (NO) free radicals [37]. Superoxide is the first reduction product of molecular oxygen, and it is an important source of hydroperoxides and deleterious free radicals [38]. Hydrogen peroxide (H_2O_2) reacts with reduced transition metals such as iron, via the Fenton reaction, to produce the highly reactive hydroxyl radical [39]. Most toxic effects are due to hydroxyl radical formation, which also initiates lipid peroxidation [39]. Some endogenous enzymes such as xanthine oxidase (XO), NO synthase, and monoamine oxidase (MAO) can directly produce ROS [36,37,40]. Normally, the ROS within the cells are neutralized by antioxidant defense mechanisms. Superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx) are the primary enzymes involved in direct elimination of ROS, whereas glutathione reductase and glucose-6-phosphate dehydrogenase are secondary antioxidant enzymes, which help in maintaining a steady concentration of glutathione and NADPH necessary for optimal functioning of the primary antioxidant enzymes [41–44]. These enzymes require micronutrients as cofactors such as selenium, iron, copper, zinc, and manganese for optimal catalytic activity and effective antioxidative defense mechanism [45]. Additionally, glutathione (GSH), iron-binding transferrin, copper-binding ceruloplasmin, α-tocopherol (Vitamin E), carotenoids, and ascorbic acid (Vitamin C) are also involved in the anti-ROS defense system [46–48]. GSH is the most important antioxidant for detoxification and elimination of environmental toxins. Oxidative stress occurs when ROS levels exceed the antioxidant capacity of a cell. These ROS are highly toxic and react with lipids, proteins and nucleic acids, and lead to cell death via apoptosis or necrosis [49].

The brain is highly vulnerable to oxidative stress due to its limited antioxidant capacity, higher energy requirement, and higher amounts of lipids and iron [50]. The brain makes up about 2% of body mass but consumes 20% of metabolic oxygen. The vast majority of energy is used by the neurons [51]. Due to the lack of glutathione-producing capacity by neurons, the brain has a limited capacity to detoxify ROS. Therefore, neurons are the first cells to be affected by the increase in ROS and shortage of antioxidants and, as a result, are most susceptible to oxidative stress. Antioxidants are required for neuronal survival during the early critical period [52]. Children are more vulnerable than adults to oxidative stress because of their naturally low glutathione levels from conception through infancy [46,53]. The risk created by this natural deficit in detoxification capacity in infants is increased by the fact that some environmental factors that induce oxidative stress are found at higher concentrations in developing infants than in their mothers, and accumulate in the placenta. Taken together, these studies suggest that the brain is highly vulnerable to oxidative stress, particularly during the early

part of development that may result in neurodevelopmental disorders such as autism. In fact, recent evidence points towards increased oxidative stress in autism.

2. Increased lipid peroxidation in autism

We have reported that lipid peroxidation is increased in the plasma of children with autism as compared to their developmentally normal, non-autistic siblings [28]. Lipid peroxidation is a chain reaction between polyunsaturated fatty acids and ROS, and it produces lipid peroxides and hydrocarbon polymers that are both highly toxic to the cell [54]. Malonyldialdehyde (MDA) is an end product of peroxidation of polyunsaturated fatty acids and related esters, and is, therefore, used as a marker of lipid peroxidation [55]. The plasma MDA contents measured by reaction with thiobarbituric acid (TBA) were higher in 13 of 15 (87%) of autistic subjects [28].

Recent reports also indicate increased levels of other lipid peroxidation markers in autism, thus confirming an increased oxidative stress in autism. For instance, Zoroglu et al. [56] have reported increased TBA-reactive substances in erythrocytes of patients with autism as compared to normal controls. Ming et al. [57] reported increased excretion of 8-isoprostane-F2alpha in the urine of children with autism. Isoprostanes are produced from the free radical oxidation of arachidonic acid through non-enzymatic oxidation of cell membrane lipids. In another study, the density of lipofuscin, a matrix of oxidized lipid and cross-linked protein that forms as a result of oxidative injury in the tissues, was observed to be greater in cortical brain areas concerned with social behavior and communication in autism [58].

3. Mechanism of oxidative stress in autism

The oxidative stress in autism may be caused by an imbalance between the generation of ROS by endogenous/exogenous pro-oxidants and the defense mechanism against ROS by antioxidants. A potential mechanism of oxidative stress in autism is shown in Fig. 1. Various factors leading to increased oxidative stress in autism are as follows.

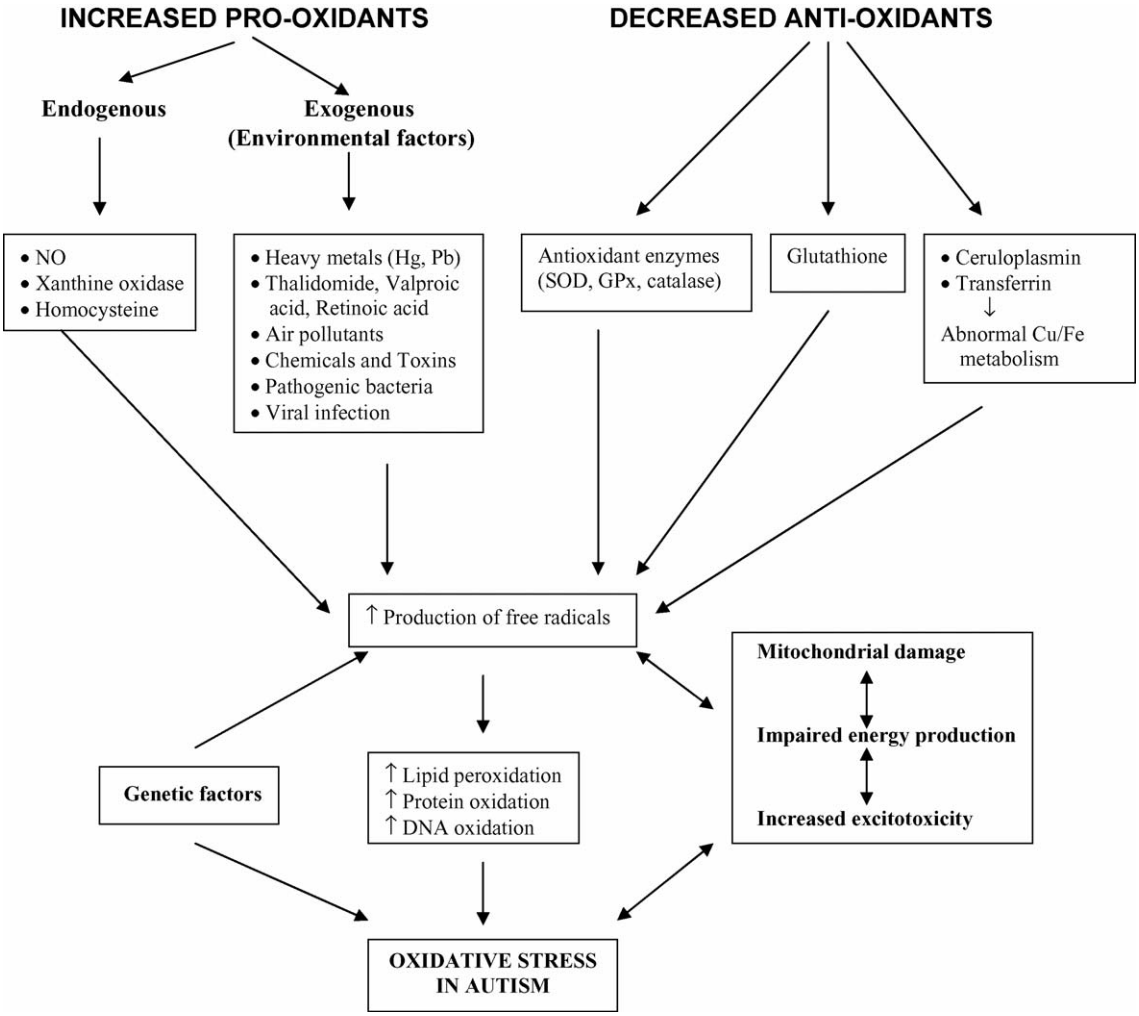

Fig. 1. Potential mechanism of oxidative stress in autism.

3.1. Alterations in antioxidant enzymes in autism

Several studies have suggested alterations in the enzymes that play a vital role in the defense mechanism against damage by ROS in autism. For instance, compared to controls, patients with autism showed decreased activity of glutathione peroxidase in plasma [59] and in erythrocytes [59,60], reduced levels of total glutathione and lower redox ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) in plasma [61], and decreased catalase [56] and SOD [59] activity in erythrocytes. In contrast, Sogut et al. [62] reported unchanged plasma SOD activity and increased GPx activity in autism.

3.2. Abnormal iron and copper metabolism in autism

Ceruloplasmin (a copper-transporting protein) and transferrin (an iron-transporting protein) are major antioxidant proteins that are synthesized in several tissues, including brain [47,48,63]. Ceruloplasmin inhibits the peroxidation of membrane lipids catalyzed by metal ions, such as iron and copper [47]. It also acts as ferroxidase and superoxide dismutase, and it protects polyunsaturated fatty acids in red blood cell membranes from active oxygen radicals [63]. Transferrin acts as an antioxidant by reducing the concentration of free ferrous ion [48]. Ferrous ion contributes to oxidative stress by catalyzing the conversion of hydrogen peroxide to highly toxic hydroxyl radicals by the Fenton reaction [39]. In addition, the Fe^{3+}-protoporphyrin (heme) group is also present in the four protein subunits of catalase enzyme [42].

We have recently reported that the levels of ceruloplasmin and transferrin are reduced in the serum of children with autism as compared to their unaffected siblings [28]. The transferrin levels were observed to be lower in 16 of 19 (84%) children with autism as compared to their unaffected siblings [28], whereas ceruloplasmin levels were lower in 13 of 19 (68%) children with autism as compared to their developmentally normal siblings [28]. It was of particular interest to observe that the levels of ceruloplasmin and transferrin were reduced more effectively in children with autism who had lost previously acquired language skills [28]. Children who had not lost language skills had levels similar to those seen in the non-autistic siblings. These results suggest that there is an altered regulation of transferrin and ceruloplasmin in a subset of children with autism. Such alterations may lead to abnormal iron and copper metabolism that may play a pathological role in autism. In fact, some preliminary studies have suggested altered serum Cu/Zn ratios in autism (reviewed in [29]).

3.3. Imbalance in homocysteine and methionine metabolism in autism

Hyperhomocysteinaemia can cause oxidative stress via a number of mechanisms such as auto-oxidation of homocysteine to form ROS [64], increased lipid peroxidation [65], and reduced production of GPx [66]. Recently, Pasca et al. [60] reported higher total homocysteine levels in plasma of children with autism as compared to control subjects. In the autistic group, a strong negative correlation was noted between homocysteine levels and glutathione peroxidase activity, suggesting an association between high levels of homocysteine and oxidative stress in autism.

Within the methionine cycle, methionine synthase, betaine homocysteine methyltransferase, and methionine adenosyltransferase are all redox-sensitive enzymes that are downregulated by oxidative stress [61]. Recently, lower concentrations of methionine, *S*-adenosylmethionine (SAM), homocysteine, cystathionine, and cysteine and higher concentrations of *S*-adenosinehomocysteine (SAH) and adenosine have been reported in the plasma of children with autism [61]. An increased vulnerability to oxidative stress and a decreased capacity for methylation (significantly lower ratio of SAM to SAH) was, therefore, suggested in autism [61].

3.4. Increased nitric oxide in autism

NO is another toxic free radical that can react with superoxide anion and generate cytotoxic peroxynitrate anions ($ONOO^-$). NO is known to affect the development and function of the central nervous system. Its role has been implicated in neurotransmitter release [67], neurite growth [68], synaptogenesis [69], memory and learning [70], and macrophage-mediated cytotoxicity [71]. The expression of inducible nitric oxide synthase (iNOS) and production of NO are also known to affect inflammatory processes [72]. The induction of iNOS is mediated by the cytokines, namely interferon (IFN)-γ, tumor necrosis factor (TNF)-α and interleukin (IL)-1β [73].

Sogut et al. [62] have reported increased NO levels in red blood cells of patients with autism and have suggested that NOS may be activated in autism. Elevated plasma levels of nitrite and nitrate in autism were also reported by Zoroglu et al. [74] and Sweeten et al. [75]. A positive correlation was observed between nitrates and IFN-γ levels in the autistic subjects, indicating that elevated plasma NO may be related to IFN-γ activity in autism [75]. Decreased activity of receptors sensitive to NO or increased oxidative stress has also been reported in autism. The cholinergic receptors known to be sensitive to NO toxicity were decreased in the cortex of patients with autism [76]. Additionally, treatment with cholinergic agonists improved behavioral abnormalities in autism [77]. In other studies, gamma aminobutyric acid (GABA) receptors that are sensitive to oxidative stress were reduced in the hippocampus of patients with autism [78,79].

3.5. Increased xanthine oxidase in autism

XO is an endogenous pro-oxidant that produces superoxide radicals during conversion of xanthine to uric acid [40]. Increased XO activity has been reported in the erythrocytes of patients with autism [56].

3.6. Mitochondrial dysfunction and abnormal energy metabolism in autism

Reactive oxygen and nitrogen species are generated endogenously during oxidative metabolism and energy production by mitochondria in the body [80]. While oxidative phosphorylation in the mitochondria generates superoxide anion, enzymatic oxidation of biogenic amines by MAO in mitochondrial outer membrane produces H_2O_2. Damaged mitochondria not only produce more oxidants, but mitochondria are also vulnerable to oxidative stress [81]. The role of mitochondria in apoptosis is also well recognized [82].

Several biochemical, anatomical and neuroradiographical studies have suggested a disturbance of energy metabolism in the brain of patients with autism [83,84]. ^{31}P-magnetic resonance spectroscopy showed increased membrane degradation and decreased synthesis of high-energy adenosine tri-phosphate (ATP) [85]. Filipek et al. [86] reported carnitine deficiency accompanied by elevations in lactate, alanine, and ammonia levels in autism, findings suggestive of mild mitochondrial dysfunction in autism. Other studies have also suggested increased lactate levels [84,87], and mitochondrial dysfunction with concomitant defects in neuronal oxidative phosphorylation in autism [88,89].

3.7. Environmental risk factors in autism

As shown in Fig. 1, prenatal or postnatal environmental exposure to pro-oxidant factors such as mercury, lead, viruses, air pollutants, toxins, thalidomide, valproic acid, and retinoic acid may act as a trigger to increase oxidative stress in autism. Increased body burdens of environmental toxins, which may induce oxidative stress, have been reported in some children with autism [10,90].

Recently, controversy has arisen about exposure to mercury from consumption of contaminated seafood during pregnancy, dental amalgams, and the mercury-based preservative thimerosal used until recently in routine childhood vaccines and flu vaccines, as a risk factor for the development of autism, especially in genetically susceptible children [7]. Mercury is a potent toxic pro-oxidant that targets the developing nervous system. An association of thimerosal-induced neurotoxicity with glutathione depletion, and a protective benefit of GSH against mercury neurotoxicity have been reported recently [91]. Another environmental factor to receive attention has been the proposed association between autism and the measles-mumps-rubella (MMR) vaccine [8,9,15,92,93]. However, results of the studies regarding the involvement of measles virus and/or the MMR vaccine in the development of autism have often been inconclusive and contradictory.

Some studies have suggested that exposure to infectious agents such as rubella or herpes virus, or toxins with associated inflammation may play a role in the development of autism [8,18,23–27]. Association has been described between autism and infections such as prenatal rubella virus [23,24] and cytomegalovirus [25,26], and postnatal herpes encephalitis [27].

3.8. Genetic susceptibility to autism

Genetic factors may also contribute in modulating the threshold for vulnerability to oxidative stress in autism. Recently, glyoxalase 1 (Glo 1) and glutathione reductase 1 (Gsr 1) have been reported to regulate anxiety-like behavior in mice [94]. The proteomic studies have also identified a single nucleotide polymorphism in glyoxalase I as an autism susceptibility factor [95]. Additionally, a functional polymorphism in the monoamine oxidase A (MAOA) promoter region has been reported to be associated with the severity of autism [96]. All these enzymes are involved in oxidative stress. Gsr maintains the levels of GSH, a major antioxidant in the brain [94]. Glo 1 uses GSH as a cofactor to detoxify cytotoxic 2-oxoaldehydes, such as methylglyoxal, that are produced by lipid peroxidation, glycation, and degradation of glycolytic intermediates [97]. MAOA catalyzes the oxidation of amine-containing neurotransmitters, such as serotonin and norepinephrine [36,37]. In another study, BTG3, a member of a family of antiproliferative genes, that plays a role in cellular differentiation and apoptosis, and is involved in cellular responses to redox changes, has been suggested as a susceptibility gene in autism [98]. These studies provide additional support for the involvement of oxidative stress in the etiology of autism.

4. Potential mechanisms that may link oxidative stress to neuronal dysfunction, clinical symptoms and pathogenesis in autism

Oxidative stress is known to be associated with premature aging of cells and can lead to tissue inflammation, damaged cell membranes, autoimmunity, and cell death [99]. Recent evidence has shown abnormalities in membrane lipid metabolism and an imbalance in immune and inflammatory responses in autism. A potential mechanism depicting association of oxidative stress in autism with membrane lipid abnormalities, immune dysregulation, inflammatory response, impaired energy metabolism, increased excitotoxicity, leading to clinical symptoms and pathology of autism is represented in Fig. 2.

4.1. Membrane lipid abnormalities in autism

Phospholipids make up the bulk of all internal and external neuronal membranes. Most neuronal membrane proteins are embedded in or attached to membrane phospholipids. The quaternary structure and function of proteins depends on the precise composition of its immediate phospholipid environment. Membrane phospholipid abnormalities have been reported in many psychiatric/behavioral disorders such as schizophrenia, dyslexia, and dyspraxia [100,101]. Recently,

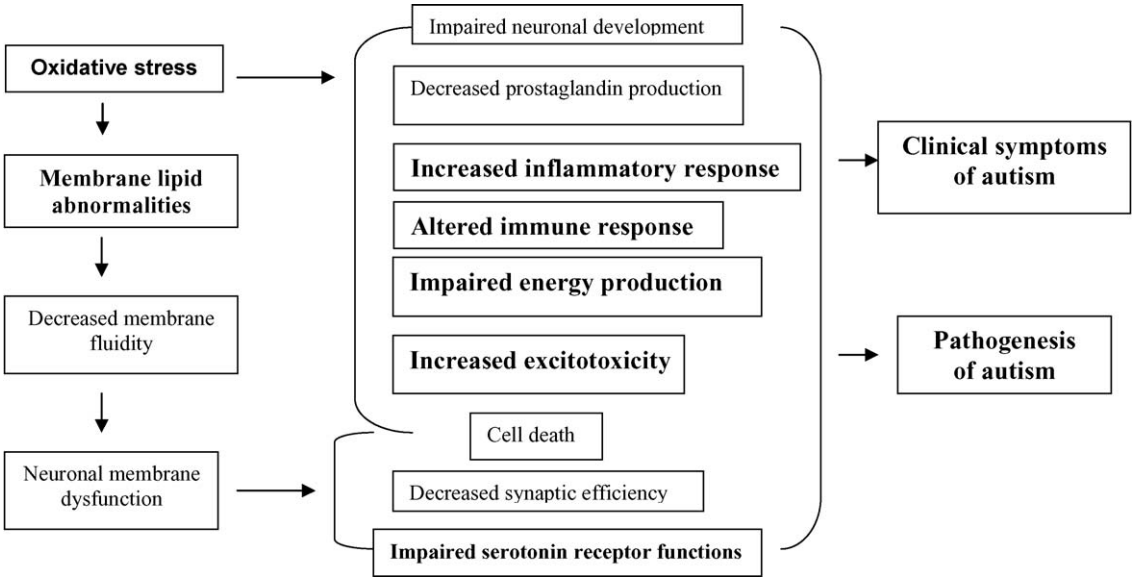

Fig. 2. Schematic depiction of potential mechanisms that may mediate neuronal dysfunction and clinical symptoms in autism.

we have reported that the phospholipid composition of the erythrocyte membrane is also altered in autism [102]. While the major phospholipids, namely phosphatidylcholine and sphingomyelin, remained unchanged, the levels of phosphatidylethanolamine (PE) were significantly lower and of phosphatidylserine (PS) were higher in the children with autism than in their unaffected siblings [102]. Further studies showed that copper (a pro-oxidant metal) selectively oxidized PE in liposomes containing brain lipids [103], indicating that abnormalities in metabolism of transitional metals may have deleterious effects in autism.

PE and PS are aminoglycerophospholipids (AGPs), i.e. glycerophospholipids containing amino groups. These lipids are found mainly on the cytoplasmic side of the membrane. During oxidative stress both in vivo [104] and in vitro [55], the normal asymmetry of biological membranes is lost, and PS and PE are externalized. It is suggested that alterations in the levels of AGP in autism may be due to increased oxidative stress.

The levels of phospholipase A_2 (PLA_2) are increased in the erythrocytes of patients with both regressive autism and classical autism/Asperger's syndrome [105]. Chromosomal linkage studies in autism also point to a locus where the PLA_2 gene (7q31) is located [3]. Therefore, this enzyme may have an important role in the etiology of autism. PLA_2 hydrolyzes the *sn*-2 fatty acids of phospholipids, giving rise to polyunsaturated fatty acids and lysophosphatidylcholine. The released fatty acids are involved in the production of prostaglandin, thromboxane, and leukotriene.

The ease with which a fatty acid can be oxidized increases with the number of double bonds in the fatty acid chain. The essential fatty acids (EFAs) of the brain are exceptionally susceptible to oxidation that can occur even when the fatty acid is in membrane phospholipids. However, its oxidation takes place at a much higher rate when the EFA is in a free form [101]. EFAs may, therefore, be particularly vulnerable to oxidation when liberated by PLA_2 [101]. A recent study has shown that polyunsaturated fatty acids are lower in the erythrocyte membranes of individuals with autism than in normal control subjects [105]. This may be due to increased oxidation of fatty acids in autism. In another study, supplementation with eicosapentaenoic acid (EPA), a major n-3 fatty acid, in patients with autism/Asperger's syndrome resulted in significantly reduced PLA_2 concentrations than in non-treated autistic subjects [105].

4.2. Decreased membrane fluidity in autism

Oxidative stress-induced production of lipid peroxides and their by-products is known to lead to the loss of membrane functions and integrity [55]. We have observed that the fluidity of the erythrocyte membrane of children with autism is lower than that of unaffected siblings [106]. These results suggest that membranes become more rigid in autism.

4.3. Immune response in autism

Several studies have suggested a link between oxidative stress and the immune response [107–109]. Changes in phagocyte functions such as adherence, chemotaxis, or TNF-α production have been reported to be associated with oxidative stress in endotoxin-induced septic shock [110]. The cytokines produced by immune cells are controlled by antioxidants with free radical-scavenging action [107]. Because immune cell functions are specially linked to ROS generation, the oxidant/antioxidant balance is essential for normal functioning of these cells. Increased serotonin levels have been reported in the blood of individuals with autism [111–114]. Given that serotonin is an immunomodulator [115,116], and most serotonin is in the gut [114,117], its elevated levels

might also lead to immune alterations and gut dysfunction in a cohort of autistic subjects.

Immune abnormalities reported in autism include decreased response to T-cell mitogens [118,119], reduced natural killer cell activity [120], depletion of CD^{4+} T helper/inducer cells [119], and increased neopterin levels in the plasma [121] and urine [122]. An imbalance of serum immunoglobulins and cytokines [123–129], autoimmunity to myelin basic proteins [130] and neuronal and glial proteins [131,132], and inappropriate antibody response to MMR vaccine [8,15,92,93,133] have been suggested to be involved in the pathogenesis of autism. Studies have also shown higher frequency of autoimmune disorders, such as rheumatoid arthritis, lupus and hypothyroidism/Hashimoto's thyroiditis in families with autistic probands than in those of healthy control subjects [20–22].

4.4. Inflammatory response in autism

A number of studies have implicated oxidative stress as a major upstream component in the signaling cascade involved in activation of redox-sensitive transcription factors and pro-inflammatory gene expression leading to inflammatory response [134,135]. Complement C3/C4 proteins [136] and alpha 1-antichymotrypsin (ACT) [137] are the positive acute phase proteins (APP) in blood that facilitate immunological and inflammatory responses**.** Levels of C3/C4 and ACT increase in the presence of inflammation and bacterial infections. Transferrin, on the other hand, is a negative APP, whose levels decrease during inflammation [48]. Our results, in fact, suggest that the serum levels of C3/C4 and ACT are higher in children with autism than in their unaffected siblings [138,139]. We have previously reported decreased transferrin levels in autism [28]. The increased levels of C3/C4 and ACT (positive APP), and the decreased levels of transferrin (negative APP), in autism suggest that inflammatory reactions may play a role in the pathogenesis of this disease.

Vargas et al. [125] have demonstrated neuroglial and innate neuroimmune system activation in autism, as evidenced by neuroinflammation in the brains, marked activation of microglia and astroglia, as well as pro-inflammatory profile of cytokines in the cerebrospinal fluid of patients with autism [125]. Cytokines and chemokines are known to play important roles as mediators of inflammatory reactions in the central nervous system, and in the neuronal-neuroglial interactions. Recently, Molloy et al. have shown increased activation of both type 1 and 2 helper T cells (Th1, Th2) arms of the adaptive immune system, with a Th2 predominance [128]. Levels of Th2 cytokines (IL-2, IL-4, IL-5, IL-13), and Th1 cytokine IFN-γ were higher without the compensatory increase in the regulatory cytokine IL-10 in peripheral blood mononuclear cells (PMNC) of children with autism than controls [128]. In other studies, IFN-γ, TNF-α and IL-β, cytokines known to be involved in production of NO, were increased in PMNC from children with autism [126,127].

Gastrointestinal symptoms and inflammatory mucosal pathology have been demonstrated in a cohort of children with autism [11–15]. In this subset of autistic children, Ashwood and Wakefield have reported increased levels of pro-inflammatory cytokines (IFN-γ, TNF-α) and reduced levels of regulatory IL-10 cytokine in peripheral blood and mucosal lymphocytes [129]. Furthermore, some studies have suggested an association between gut inflammation and NO-dependent oxidative injury [29]. It is possible that increased NO levels in autism may also be responsible for the gastrointestinal abnormalities observed in subset of individuals with autism [29]. Cytokines and products of immune activation have also been suggested to contribute to other common features of autism such as mood and sleep disturbance [129]. Collectively, these studies suggest that inflammatory phenomenon and immune dysregulation may contribute to the development and pathogenesis of autism.

4.5. Increased excitotoxicity in autism

Excitotoxicity has been suggested as a contributing factor to oxidative stress, as well as a result of oxidative stress. Glutamic acid decarboxylase (GAD) that converts glutamate to GABA, glutamine synthase, glutamate transporter, and GABA receptors are vulnerable to oxidative stress (reviewed in [29]). Increased extra-cellular glutamate and reduced GABA are known to increase excitotoxicity. In autism, reduced GAD [140], higher plasma glutamate and reduced glutamine [141] have been reported. These findings are suggestive of increased excitotoxicity in autism.

5. Potential antioxidant therapy in autism

Several double-blind, placebo-controlled therapeutic trials of the use of potent antioxidants such as Vitamin C, carnosine, zinc, reduced glutathione, fish oil (rich in EPA), melatonin and Vitamin B6 in combination with magnesium in autism are ongoing [29]. In double-blind, placebo-controlled clinical trials, treatment with high dose Vitamin C [142] or carnosine [143] or combined Vitamin B6 and magnesium [144,145] improved the behavior of individuals with autism. Additionally, melatonin has been reported to be useful in the treatment of sleep disorders in autism [146].

6. Conclusion

Extensive evidence suggests increased oxidative stress in autism with likely contributions from environment, genetic and immunological factors. Increased oxidative stress in autism may be due to (a) increased production of endogenous pro-oxidants (such as NO, xanthine oxidase, homocysteine) or environmental pro-oxidants, or (b) deficiencies of antioxidants (ceruloplasmin, transferrin, SOD, GPx, catalase, reduced glutathione), or (c) both. Reduced levels of serum

ceruloplasmin (a copper-transport protein) and transferrin (an iron-transport protein) in autism suggest that metabolism of iron and copper (pro-oxidant components of oxidative stress) may be abnormal in autism. Increased oxidative stress may lead to membrane lipid abnormalities, mitochondrial dysfunction, excitotoxicity, inflammation, and immune dysregulation in autism. These abnormalities might contribute to behavioral abnormalities, sleep disorder, and gastrointestinal disturbances in autism. Preliminary results of some of clinical trials have suggested improved behavior in individuals with autism who receive antioxidant therapy.

Acknowledgements

This work was supported in part by funds from New York State Office of Mental Retardation and Developmental Disabilities, a Cure Autism Now Foundation pilot grant, and a New York State Legislative Grant for Autism Research.

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