Research has always been one of the core initiatives of SafeMinds. In 1999, when founding members of the organization recognized the extensive overlap between the symptoms of mercury toxicity and autism, they published an extensive literature review documenting these findings. In so doing, SafeMinds brought the issue of environmental factors in the etiology of autism to national and international attention. Since that time, SafeMinds has diligently worked to move the science forward by continuing to write and publish peer-reviewed research articles, network with leading scientists in the fields of toxicology and autism research, and directly fund strategic research necessary to advance our understanding of the role of environment in the development of autism spectrum disorders. The overall goals of SafeMinds research initiatives are to elucidate mechanisms of injury, identify target organ systems prone to injury and the manifestations of those injuries, to better understand the role of genetic susceptibility and most importantly, how to effectively restore health to those with autism spectrum disorders. Important research findings are rapidly communicated to the public in an effort to raise awareness and prevent future harm. To date, SafeMinds efforts are responsible for the publication of over 20 research articles and investment of $1.3 million dollars into research. The following are our findings in order by date.

Med Hypotheses. 2001 Apr;56(4):462-71.

Autism: a novel form of mercury poisoning.

Bernard S, Enayati A, Redwood L, Roger H, Binstock T.

ARC Research, Cranford, New Jersey 07901, USA.

Abstract

Autism is a syndrome characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movements, and sensory dysfunction. Recent epidemiological studies suggest that autism may affect 1 in 150 US children. Exposure to mercury can cause immune, sensory, neurological, motor, and behavioral dysfunctions similar to traits defining or associated with autism, and the similarities extend to neuroanatomy, neurotransmitters, and biochemistry. Thimerosal, a preservative added to many vaccines, has become a major source of mercury in children who, within their first two years, may have received a quantity of mercury that exceeds safety guidelines. A review of medical literature and US government data suggests that: (i) many cases of idiopathic autism are induced by early mercury exposure from thimerosal; (ii) this type of autism represents an unrecognized mercurial syndrome; and (iii) genetic and non-genetic factors establish a predisposition whereby thimerosal's adverse effects occur only in some children.

PMID: 11339848
http://www.ncbi.nlm.nih.gov/pubmed/11339848

Neurotoxicology. 2001 Oct;22(5):691-7.

Predicted mercury concentrations in hair from infant immunizations: cause for concern.

Redwood L, Bernard S, Brown D.

Coalition for Safe Minds, Cranford, NJ 07016, USA. tlredwood@mindspring.com

Abstract

Mercury (Hg) is considered one of the worlds most toxic metals. Current thinking suggests that exposure to mercury occurs primarily from seafood contamination and rare catastrophic events. Recently, another common source of exposure has been identified. Thimerosal (TMS), a preservative found in many infant vaccines, contains 49.6% ethyl mercury (EtHg) by weight and typically contributes 25 microg of EtHg per dose of infant vaccine. As part of an ongoing review, the Food and Drug Administration (FDA) announced in 1999 that infants who received multiple TMS-preserved vaccines may have been exposed to cumulative Hg in excess of Federal safety guidelines. According to the centers for disease control (CDC) recommended immunization schedule, infants may have been exposed to 12.5 microg Hg at birth, 62.5 microg EtHg at 2 months, 50 microg EtHg at 4 months, 62.5 microg EtHg at 6 months, and 50 microg EtHg at approximately 18 months, for a total of 237.5 microg EtHg during the first 18 months of life, if all TMS-containing vaccines were administered. Neurobehavioral alterations, especially to the more susceptible fetus and infant, are known to occur after relatively low dose exposures to organic mercury compounds. In effort, to further elucidate the levels of ethyl mercury resulting from exposure to vaccinal TMS, we estimated hair Hg concentrations expected to result from the recommended CDC schedule utilizing a one compartment pharmacokinetic model. This model was developed to predict hair concentrations from acute exposure to methymercury (MeHg) in fish. Modeled hair Hg concentrations in infants exposed to vaccinal TMS are in excess of the Environmental Protection Agency (EPA) safety guidelines of 1 ppm for up to 365 days, with several peak concentrations within this period. More sensitive individuals and those with additional sources of exposure would have higher Hg concentrations. Given that exposure to low levels of mercury during critical stages of development has been associated with neurological disorders in children, including ADD, learning difficulties, and speech delays, the predicted hair Hg concentration resulting from childhood immunizations is cause for concern. Based on these findings, the impact which vaccinal mercury has had on the health of American children warrants further investigation.

PMID: 11770890
http://www.ncbi.nlm.nih.gov/pubmed/11770890

Mol Psychiatry. 2002;7 Suppl 2:S42-3.

The role of mercury in the pathogenesis of autism.

Bernard S, Enayati A, Roger H, Binstock T, Redwood L.

Safe Minds, 14 Commerce Drive, Cranford, NJ 07901, USA. sbernard@nac.net

Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder of unknown etiology in most cases. Studies of monozygotic twins report an average 60% concordance rate, indicating a role for both genetic and environmental factors in disease expression. Recent reviews in environmental health have suggested that early exposure to hazardous substances may underlie some cases of neurodevelopmental disorders, including ADHD, learning disabilities, and speech/language difficulties. In 1999, thimerosal used as a vaccine preservative was identified as a widespread source of organic mercury exposure in infants. Mercury (Hg), a heavy metal, is considered highly neurotoxic. The amount of mercury in vaccines, while small, exceeded USEPA safety guidelines on a cumulative basis. Certain individuals may exhibit severe adverse reactions to low doses of Hg which are otherwise largely benign to the majority of those exposed. Some individuals with idiopathic autism spectrum disorder may represent such a sensitive population. As summarized in this paper, disease characteristics suggest this possibility: (a) ASD traits are known to arise from mercury exposure; (b) onset of ASD symptoms is temporally associated with administration of immunizations; (c) the reported increase in the prevalence of autism in the 1990s closely follows the introduction of two mercurycontaining vaccines; and (d) elevated mercury has been detected in biological samples of autistic patients. Since ASD may now affect as many as one in 150 US children, and since thimerosal is still used in many poducts worldwide, confirmation of thimerosal as an environmental agent in autism pathogenesis has important societal and patient implications.

PMID:12142947 **Free Article**
http://www.ncbi.nlm.nih.gov/pubmed/12142947

Int J Toxicol. 2003 Jul-Aug;22(4):277-85.

Reduced levels of mercury in first baby haircuts of autistic children.

Holmes AS, Blaxill MF, Haley BE.

SafeMinds, Cambridge, Massachusetts, USA.

Abstract

Reported rates of autism have increased sharply in the United States and the United Kingdom. One possible factor underlying these increases is increased exposure to mercury through thimerosal-containing vaccines, but vaccine exposures need to be evaluated in the context of cumulative exposures during gestation and early infancy. Differential rates of postnatal mercury elimination may explain why similar gestational and infant exposures produce variable neurological effects. First baby haircut samples were obtained from 94 children diagnosed with autism using Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM IV) criteria and 45 age- and gender-matched controls. Information on diet, dental amalgam fillings, vaccine history, Rho D immunoglobulin administration, and autism symptom severity was collected through a maternal survey questionnaire and clinical observation. Hair mercury levels in the autistic group were 0.47 ppm versus 3.63 ppm in controls, a significant difference. The mothers in the autistic group had significantly higher levels of mercury exposure through Rho D immunoglobulin injections and amalgam fillings than control mothers. Within the autistic group, hair mercury levels varied significantly across mildly, moderately, and severely autistic children, with mean group levels of 0.79, 0.46, and 0.21 ppm, respectively. Hair mercury levels among controls were significantly correlated with the number of the mothers' amalgam fillings and their fish consumption as well as exposure to mercury through childhood vaccines, correlations that were absent in the autistic group. Hair excretion patterns among autistic infants were significantly reduced relative to control. These data cast doubt on the efficacy of traditional hair analysis as a measure of total mercury exposure in a subset of the population. In light of the biological plausibility of mercury's role in neurodevelopmental disorders, the present study provides further insight into one possible mechanism by which early mercury exposures could increase the risk of autism.

PMID: 12933322
http://www.ncbi.nlm.nih.gov/pubmed/12933322

Toxicol Sci. 2003 Aug;74(2):361-8. Epub 2003 May 28.

Thimerosal induces DNA breaks, caspase-3 activation, membrane damage, and cell death in cultured human neurons and fibroblasts.

Baskin DS, Ngo H, Didenko VV.

Department of Neurosurgery, Baylor College of Medicine, 6560 Fannin Suite 944, Houston, Texas 77030, USA. dbaskin@tmh.tmc.edu

Abstract

Thimerosal is an organic mercurial compound used as a preservative in biomedical preparations. Little is known about the reactions of human neuronal and skin cells to its micro- and nanomolar concentrations, which can occur after using thimerosal-containing products. A useful combination of fluorescent techniques for the assessment of thimerosal toxicity is introduced. Short-term thimerosal toxicity was investigated in cultured human cerebral cortical neurons and in normal human fibroblasts. Cells were incubated with 125-nM to 250-microM concentrations of thimerosal for 45 min to 24 h. A 4', 6-diamidino-2-phenylindole dihydrochloride (DAPI) dye exclusion test was used to identify nonviable cells and terminal transferase-based nick-end labeling (TUNEL) to label DNA damage. Detection of active caspase-3 was performed in live cell cultures using a cell-permeable fluorescent caspase inhibitor. The morphology of fluorescently labeled nuclei was analyzed. After 6 h of incubation, the thimerosal toxicity was observed at 2 microM based on the manual detection of the fluorescent attached cells and at a 1-microM level with the more sensitive GENios Plus Multi-Detection Microplate Reader with Enhanced Fluorescence. The lower limit did not change after 24 h of incubation. Cortical neurons demonstrated higher sensitivity to thimerosal compared to fibroblasts. The first sign of toxicity was an increase in membrane permeability to DAPI after 2 h of incubation with 250 microM thimerosal. A 6-h incubation resulted in failure to exclude DAPI, generation of DNA breaks, caspase-3 activation, and development of morphological signs of apoptosis. We demonstrate that thimerosal in micromolar concentrations rapidly induce membrane and DNA damage and initiate caspase-3-dependent apoptosis in human neurons and fibroblasts. We conclude that a proposed combination of fluorescent techniques can be useful in analyzing the toxicity of thimerosal.

PMID: 12773768 **Free Article**
http://www.ncbi.nlm.nih.gov/pubmed/12773768

Mol Psychiatry. 2004 Sep;9(9):833-45.

Neurotoxic effects of postnatal thimerosal are mouse strain dependent.

Hornig M, Chian D, Lipkin WI.

Jerome L and Dawn Greene Infectious Disease Laboratory, Department of Epidemiology, Mailman School of Public Health, Columbia University, New York, NY 10032, USA. mady.hornig@columbia.edu

Abstract

The developing brain is uniquely susceptible to the neurotoxic hazard posed by mercurials. Host differences in maturation, metabolism, nutrition, sex, and autoimmunity influence outcomes. How population-based variability affects the safety of the ethylmercury-containing vaccine preservative, thimerosal, is unknown. Reported increases in the prevalence of autism, a highly heritable neuropsychiatric condition, are intensifying public focus on environmental exposures such as thimerosal. Immune profiles and family history in autism are frequently consistent with autoimmunity. We hypothesized that autoimmune propensity influences outcomes in mice following thimerosal challenges that mimic routine childhood immunizations. Autoimmune disease-sensitive SJL/J mice showed growth delay; reduced locomotion; exaggerated response to novelty; and densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters. Strains resistant to autoimmunity, C57BL/6J and BALB/cJ, were not susceptible. These findings implicate genetic influences and provide a model for investigating thimerosal-related neurotoxicity.

PMID: 15184908
http://www.ncbi.nlm.nih.gov/pubmed/15184908

Med Hypotheses. 2004;62(5):788-94.

Thimerosal and autism? A plausible hypothesis that should not be dismissed.

Blaxill MF, Redwood L, Bernard S.

Safe Minds, 14 Commerce Drive, PH Cranford, New Jersey 07016, USA. blaxill@comcast.net

Abstract

The autism-mercury hypothesis first described by Bernard et al. has generated much interest and controversy. The Institute of Medicine (IOM) reviewed the connection between mercury-containing vaccines and neurodevelopmental disorders, including autism. They concluded that the hypothesis was biologically plausible but that there was insufficient evidence to accept or reject a causal connection and recommended a comprehensive research program. Without citing new experimental evidence, a number of observers have offered opinions on the subject, some of which reject the IOM's conclusions. In a recent review, Nelson and Bauman argue that a link between the preservative thimerosal, the source of the mercury in childhood vaccines, is improbable. In their defense of thimerosal, these authors take a narrow view of the original hypothesis, provide no new evidence, and rely on selective citations and flawed reasoning. We provide evidence here to refute the Nelson and Bauman critique and to defend the autism-mercury hypothesis.

PMID: 15082108
http://www.ncbi.nlm.nih.gov/pubmed/15082108

Mol Psychiatry. 2004 Apr;9(4):358-70.

Activation of methionine synthase by insulin-like growth factor-1 and dopamine: a target for neurodevelopmental toxins and thimerosal.

Waly M, Olteanu H, Banerjee R, Choi SW, Mason JB, Parker BS, Sukumar S, Shim S, Sharma A, Benzecry JM, Power-Charnitsky VA, Deth RC.

Department of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115, USA.

Abstract

Methylation events play a critical role in the ability of growth factors to promote normal development. Neurodevelopmental toxins, such as ethanol and heavy metals, interrupt growth factor signaling, raising the possibility that they might exert adverse effects on methylation. We found that insulin-like growth factor-1 (IGF-1)- and dopamine-stimulated methionine synthase (MS) activity and folate-dependent methylation of phospholipids in SH-SY5Y human neuroblastoma cells, via a PI3-kinase- and MAP-kinase-dependent mechanism. The stimulation of this pathway increased DNA methylation, while its inhibition increased methylation-sensitive gene expression. Ethanol potently interfered with IGF-1 activation of MS and blocked its effect on DNA methylation, whereas it did not inhibit the effects of dopamine. Metal ions potently affected IGF-1 and dopamine-stimulated MS activity, as well as folate-dependent phospholipid methylation: Cu(2+) promoted enzyme activity and methylation, while Cu(+), Pb(2+), Hg(2+) and Al(3+) were inhibitory. The ethylmercury-containing preservative thimerosal inhibited both IGF-1- and dopamine-stimulated methylation with an IC(50) of 1 nM and eliminated MS activity. Our findings outline a novel growth factor signaling pathway that regulates MS activity and thereby modulates methylation reactions, including DNA methylation. The potent inhibition of this pathway by ethanol, lead, mercury, aluminum and thimerosal suggests that it may be an important target of neurodevelopmental toxins.

PMID: 14745455
http://www.ncbi.nlm.nih.gov/pubmed/14745455

Public Health Rep. 2004 Nov-Dec;119(6):536-51.

What's going on? The question of time trends in autism.

Blaxill MF.

SafeMinds, Cranford, NJ, USA. blaxill.mark@bcg.com

Abstract

Increases in the reported prevalence of autism and autistic spectrum disorders in recent years have fueled concern over possible environmental causes. The author reviews the available survey literature and finds evidence of large increases in prevalence in both the United States and the United Kingdom that cannot be explained by changes in diagnostic criteria or improvements in case ascertainment. Incomplete ascertainment of autism cases in young child populations is the largest source of predictable bias in prevalence surveys; however, this bias has, if anything, worked against the detection of an upward trend in recent surveys. Comparison of autism rates by year of birth for specific geographies provides the strongest basis for trend assessment. Such comparisons show large recent increases in rates of autism and autistic spectrum disorders in both the U.S. and the U.K. Reported rates of autism in the United States increased from < 3 per 10,000 children in the 1970s to > 30 per 10,000 children in the 1990s, a 10-fold increase. In the United Kingdom, autism rates rose from < 10 per 10,000 in the 1980s to roughly 30 per 10,000 in the 1990s. Reported rates for the full spectrum of autistic disorders rose from the 5 to 10 per 10,000 range to the 50 to 80 per 10,000 range in the two countries. A precautionary approach suggests that the rising incidence of autism should be a matter of urgent public concern.

PMID: 1497666 **Free Article**
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1497666/

Neurotoxicology. 2006 Sep;27(5):671-84. Epub 2006 Mar 28.

Autism and environmental genomics.

Herbert MR, Russo JP, Yang S, Roohi J, Blaxill M, Kahler SG, Cremer L, Hatchwell E.

Pediatric Neurology, Massachusetts General Hospital, Harvard Medical School, USA.
mherbert1@partners.org

Abstract

Autism spectrum disorders (ASD) are defined by behavior and diagnosed by clinical history and observation but have no biomarkers and are presumably, etiologically and biologically heterogeneous. Given brain abnormalities and high monozygotic concordance, ASDs have been framed as neurobiologically based and highly genetic, which has shaped the research agenda and in particular criteria for choosing candidate ASD genes. Genetic studies to date have not uncovered genes of strong effect, but a move toward "genetic complexity" at the neurobiological level may not suffice, as evidence of systemic abnormalities (e.g. gastrointestinal and immune), increasing rates and less than 100% monozygotic concordance support a more inclusive reframing of autism as a multisystem disorder with genetic influence and environmental contributors. We review this evidence and also use a bioinformatic approach to explore the possibility that "environmentally responsive genes" not specifically associated with the nervous system, but potentially associated with systemic changes in autism, have not hitherto received sufficient attention in autism genetics investigations. We overlapped genes from NIEHS Environmental Genome Project, the Comparative Toxicogenomics Database, and the SeattleSNPs database of genes relevant to the human immune and inflammatory response with linkage regions identified in published autism genome scans. We identified 135 genes in overlap regions, of which 56 had never previously been studied in relation to autism and 47 had functional SNPs (in coding regions). Both our review and the bioinformatics exercise support the expansion of criteria for evaluating the relevance of genes to autism risk to include genes related to systemic impact and environmental responsiveness. This review also suggests the utility of environmental genomic resources in highlighting the potential relevance of particular genes within linkage regions. Environmental responsiveness and systems impacts consistent with system-wide findings in autism are thus supported as important considerations in identifying the numerous and complex modes of gene-environment interaction in autism.

PMID: 16644012
http://www.ncbi.nlm.nih.gov/pubmed/16644012

Neurotoxicology. 2006 Sep;27(5):685-92. Epub 2006 Jun 16.

Cultured lymphocytes from autistic children and non-autistic siblings up-regulate heat shock protein RNA in response to thimerosal challenge.

Walker SJ, Segal J, Aschner M.

Department of Physiology and Pharmacology, Wake Forest University School of Medicine, Winston-Salem, NC 27156, USA. swalker@wfubmc.edu

Abstract

There are reports suggesting that some autistic children are unable to mount an adequate response following exposure to environmental toxins. This potential deficit, coupled with the similarity in clinical presentations of autism and some heavy metal toxicities, has led to the suggestion that heavy metal poisoning might play a role in the etiology of autism in uniquely susceptible individuals. Thimerosal, an anti-microbial preservative previously added routinely to childhood multi-dose vaccines, is composed of 49.6% ethyl mercury. Based on the levels of this toxin that children receive through routine immunization schedules in the first years of life, it has been postulated that thimerosal may be a potential triggering mechanism contributing to autism in susceptible individuals. One potential risk factor in these individuals may be an inability to adequately up-regulate metallothionein (MT) biosynthesis in response to presentation of a heavy metal challenge. To investigate this hypothesis, cultured lymphocytes (obtained from the Autism Genetic Resource Exchange, AGRE) from autistic children and non-autistic siblings were challenged with either 10 microM ethyl mercury, 150 microM zinc, or fresh media (control). Following the challenge, total RNA was extracted and used to query "whole genome" DNA microarrays. Cultured lymphocytes challenged with zinc responded with an impressive up-regulation of MT transcripts (at least nine different MTs were over-expressed) while cells challenged with thimerosal responded by up-regulating numerous heat shock protein transcripts, but not MTs. Although there were no apparent differences between autistic and non-autistic sibling responses in this very small sampling group, the differences in expression profiles between those cells treated with zinc versus thimerosal were dramatic. Determining cellular response, at the level of gene expression, has important implications for the understanding and treatment of conditions that result from exposure to neurotoxic compounds.

PMID: 16870260
http://www.ncbi.nlm.nih.gov/pubmed/16870260

FASEB J. 2009 Aug;23(8):2374-83. doi: 10.1096/fj.08-128926. Epub 2009 Mar 23.

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

James SJ, Rose S, Melnyk S, Jernigan S, Blossom S, Pavliv O, Gaylor DW.

Department of Pediatrics, University of Arkansas for Medical Sciences, Arkansas Children's Hospital Research Institute, 1120 Marshall St., Little Rock, AR 72202, USA. jamesjill@uams.edu

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 pro-oxidant conditions.

PMID: 2717775 **Free Article**
http://www.ncbi.nlm.nih.gov/pubmed/19307255

Expert Opin Pharmacother. 2009 Sep;10(13):2127-43. doi: 10.1517/14656560903107789.

Autism: an emerging 'neuroimmune disorder' in search of therapy.

Theoharides TC, Kempuraj D, Redwood L.

Molecular Immunopharmacology and Drug Discovery Laboratory, Tufts University School of Medicine, Tufts Medical Center, Department of Pharmacology, Boston, MA 02111, USA. theoharis.theoharides@tufts.edu

BACKGROUND:

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by difficulties in communication and by repetitive and stereotypic behaviors, as well as by social impairment, attention, cognitive, and learning defects. ASDs present in early childhood and their prevalence has increased significantly to 1/150 children. Despite a number of theories, the actual reasons for this increase are still not clear. There is no reliable screening test, and no definite pathogenesis or curative therapy. Consequently, there is a major gap hampering development of effective treatments.

OBJECTIVE:

To review recent publications on ASDs pathogenesis and treatment with emphasis on neuroimmune processes and new therapeutic approaches.

METHODS:

Mostly original papers (450) on epidemiology, possible pathogenesis or treatment of ASDs in Medline from 1990 to May 2009 were reviewed. All authors contributed to this review.

RESULTS/CONCLUSION:

Increased oxidative stress and immune dysregulation are present in ASDs. Mast-cell activation may contribute to gut-blood-brain barrier disruption and brain inflammation. No effective treatments have emerged. Well-designed clinical trials with nonpsychotropic drugs were few and ASD characteristics varied considerably, making conclusions difficult. Psychotropic drugs are often used for stereotypic and aggressive behaviors. Unique combinations with antioxidant and anti-inflammatory flavonoids hold promise. New potential translational research areas and possible treatments are suggested.

PMID: 19640207
http://www.ncbi.nlm.nih.gov/pubmed/19640207

Acta Neurobiol Exp (Wars). 2010;70(2):147-64.

Influence of pediatric vaccines on amygdala growth and opioid ligand binding in rhesus macaque infants: A pilot study.

Hewitson L, Lopresti BJ, Stott C, Mason NS, Tomko J.

Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA. lch1@pitt.edu

Comment in

- Autism and the amygdala: commentary on Hewitson and coauthors (2010). [Acta Neurobiol Exp (Wars). 2011]

Abstract

This longitudinal, case-control pilot study examined amygdala growth in rhesus macaque infants receiving the complete US childhood vaccine schedule (1994-1999). Longitudinal structural and functional neuroimaging was undertaken to examine central effects of the vaccine regimen on the developing brain. Vaccine-exposed and saline-injected control infants underwent MRI and PET imaging at approximately 4 and 6 months of age, representing two specific timeframes within the vaccination schedule. Volumetric analyses showed that exposed animals did not undergo the maturational changes over time in amygdala volume that was observed in unexposed animals. After controlling for left amygdala volume, the binding of the opioid antagonist [(11)C]diprenorphine (DPN) in exposed animals remained relatively constant over time, compared with unexposed animals, in which a significant decrease in [(11)C]DPN binding occurred. These results suggest that maturational changes in amygdala volume and the binding capacity of [(11)C]DPN in the amygdala was significantly altered in infant macaques receiving the vaccine schedule. The macaque infant is a relevant animal model in which to investigate specific environmental exposures and structural/functional neuroimaging during neurodevelopment.

PMID: 20628439 **Free Article**
http://www.ncbi.nlm.nih.gov/pubmed/20628439

J Toxicol Environ Health A. 2010;73(19):1298-313. doi: 10.1080/15287394.2010.484709.

Delayed acquisition of neonatal reflexes in newborn primates receiving a thimerosal-containing hepatitis B vaccine: influence of gestational age and birth weight.

Hewitson L, Houser LA, Stott C, Sackett G, Tomko JL, Atwood D, Blue L, White ER.

Department of Obstetrics and Gynecology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA. lch1@pitt.edu

Abstract

This study examined whether acquisition of neonatal reflexes in newborn rhesus macaques was influenced by receipt of a single neonatal dose of hepatitis B vaccine containing the preservative thimerosal (Th). Hepatitis B vaccine containing a weight-adjusted Th dose was administered to male macaques within 24 h of birth (n = 13). Unexposed animals received saline placebo (n = 4) or no injection (n = 3). Infants were tested daily for acquisition of nine survival, motor, and sensorimotor reflexes. In exposed animals there was a significant delay in the acquisition of root, snout, and suck reflexes, compared with unexposed animals. No neonatal responses were significantly delayed in unexposed animals. Gestational age (GA) and birth weight (BW) were not significantly correlated. Cox regression models were used to evaluate main effects and interactions of exposure with BW and GA as independent predictors and time-invariant covariates. Significant main effects remained for exposure on root and suck when controlling for GA and BW, such that exposed animals were relatively delayed in time-to-criterion. Interaction models indicated there were various interactions between exposure, GA, and BW and that inclusion of the relevant interaction terms significantly improved model fit. This, in turn, indicated that lower BW and/or lower GA exacerbated the adverse effects following vaccine exposure. This primate model provides a possible means of assessing adverse neurodevelopmental outcomes from neonatal Th-containing hepatitis B vaccine exposure, particularly in infants of lower GA or BW. The mechanisms underlying these effects and the requirements for Th requires further study.

PMID: 20711932
http://www.ncbi.nlm.nih.gov/pubmed/20711932

J Neuroinflammation. 2010 Mar 11;7:20. doi: 10.1186/1742-2094-7-20.

Mercury induces inflammatory mediator release from human mast cells.

Kempuraj D, Asadi S, Zhang B, Manola A, Hogan J, Peterson E, Theoharides TC.

Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine and Tufts Medical Center, Boston, MA 02111, USA.

BACKGROUND:

Mercury is known to be neurotoxic, but its effects on the immune system are less well known. Mast cells are involved in allergic reactions, but also in innate and acquired immunity, as well as in inflammation. Many patients with Autism Spectrum Disorders (ASD) have "allergic" symptoms; moreover, the prevalence of ASD in patients with mastocytosis, characterized by numerous hyperactive mast cells in most tissues, is 10-fold higher than the general population suggesting mast cell involvement. We, therefore, investigated the effect of mercuric chloride (HgCl2) on human mast cell activation.

METHODS:

Human leukemic cultured LAD2 mast cells and normal human umbilical cord blood-derived cultured mast cells (hCBMCs) were stimulated by HgCl2 (0.1-10 microM) for either 10 min for beta-hexosaminidase release or 24 hr for measuring vascular endothelial growth factor (VEGF) and IL-6 release by ELISA.

RESULTS:

HgCl2 induced a 2-fold increase in beta-hexosaminidase release, and also significant VEGF release at 0.1 and 1 microM (311 +/- 32 pg/106 cells and 443 +/- 143 pg/106 cells, respectively) from LAD2 mast cells compared to control cells (227 +/- 17 pg/106 cells, $n = 5$, $p < 0.05$). Addition of HgCl2 (0.1 microM) to the proinflammatory neuropeptide substance P (SP, 0.1 microM) had synergestic action in inducing VEGF from LAD2 mast cells. HgCl2 also stimulated significant VEGF release (360 +/- 100 pg/106 cells at 1 microM, $n = 5$, $p < 0.05$) from hCBMCs compared to control cells (182 +/- 57 pg/106 cells), and IL-6 release (466 +/- 57 pg/106 cells at 0.1 microM) compared to untreated cells (13 +/- 25 pg/106 cells, $n = 5$, $p < 0.05$). Addition of HgCl2 (0.1 microM) to SP (5 microM) further increased IL-6 release.

CONCLUSIONS:

HgCl2 stimulates VEGF and IL-6 release from human mast cells. This phenomenon could disrupt the blood-brain-barrier and permit brain inflammation. As a result, the findings of the present study provide a biological mechanism for how low levels of mercury may contribute to ASD pathogenesis.

The electronic version of this article can be found at: http://www.jneuroinflammation.com/content/7/1/20

Int J Immunopathol Pharmacol. 2010 Oct-Dec;23(4):1015-20.

Luteolin and thiosalicylate inhibit HgCl(2) and thimerosal-induced VEGF release from human mast cells.

Asadi S, Zhang B, Weng Z, Angelidou A, Kempuraj D, Alysandratos KD, Theoharides TC.

Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine and Tufts Medical Center, Boston, MA 02111, USA.

Abstract

HgCl2 is a known environemental neurotoxin, but is also used as preservative in vaccines as thimerosal containing ethyl mercury covalently linked to thiosalicylate. We recently reported that mercury choloride (HgCl(2)) can stimulate human mast cells to release vascular endothelial growth factor (VEGF), which is also vasoactive and pro-inflammatory. Here we show that thimerosal induces significant VEGF release from human leukemic cultured LAD2 mast cells (at 1 microM 326 ± 12 pg/106 cells and 335.5 ± 12 pg/106 cells at 10 microM) compared to control cells (242 ± 21 pg/106 cells, n=5, p less than 0.05); this effect is weaker than that induced by HgCl2 at 10 microM (448 ± 14 pg/106 cells) (n=3, p less than 0.05). In view of this finding, we hypothesize that the thiosalicylate component of thimerosal may have an inhibitory effect on VEGF release. Thimerosal (10 microM) added together with the peptide Substance P (SP) at 2 microM, used as a positive control, reduced VEGF release by 90 percent. Methyl thiosalicylate (1 or 10 microM) added with either SP or HgCl2 (10 microM) inhibited VEGF release by 100 percent, while sodium salicylate or ibuprofen had no effect. Pretreatment for 10 min with the flavonoid luteolin (0.1 mM) before HgCl2 or thimerosal compeletly blocked their effect. Luteolin and methyl thiosalicylate may be useful in preventing mercury-induced toxicity.

PMID: 21244751
http://www.ncbi.nlm.nih.gov/pubmed/21244751

PLoS One. 2012;7(12):e49767. doi: 10.1371/journal.pone.0049767. Epub 2012 Dec 17.

Stimulated human mast cells secrete mitochondrial components that have autocrine and paracrine inflammatory actions.

Zhang B, Asadi S, Weng Z, Sismanopoulos N, Theoharides TC.

Molecular Immunopharmacology and Drug Discovery Laboratory, Department of Molecular Physiology and Pharmacology, Tufts University School of Medicine, Boston, Massachusetts, United States of America.

Abstract

Mast cells are hematopoietically-derived tissue immune cells that participate in acquired and innate immunity, as well as in inflammation through release of many chemokines and cytokines, especially in response to the pro-inflammatory peptide substance P (SP). Inflammation is critical in the pathogenesis of many diseases, but the trigger(s) is often unknown. We investigated if mast cell stimulation leads to secretion of mitochondrial components and whether these could elicit autocrine and/or paracrine inflammatory effects. Here we show that human LAD2 mast cells stimulated by IgE/anti-IgE or by the SP led to secretion of mitochondrial particles, mitochondrial (mt) mtDNA and ATP without cell death. Mitochondria purified from LAD2 cells and, when mitochondria added to mast cells trigger degranulation and release of histamine, PGD(2), IL-8, TNF, and IL-1β. This stimulatory effect is partially inhibited by an ATP receptor antagonist and by DNAse. These results suggest that the mitochondrial protein fraction may also contribute. Purified mitochondria also stimulate IL-8 and vascular endothelial growth factor (VEGF) release from cultured human keratinocytes, and VEGF release from primary human microvascular endothelial cells. In order to investigate if mitochondrial components could be secreted in vivo, we injected rats intraperiotoneally (ip) with compound 48/80, which mimicks the action of SP. Peritoneal mast cells degranulated and mitochondrial particles were documented by transimission electron microscopy outside the cells. We also wished to investigate if mitochondrial components secreted locally could reach the systemic circulation. Administration ip of mtDNA isolated from LAD2 cells in rats was detected in their serum within 4 hr, indicating that extravascular mtDNA could enter the systemic circulation. Secretion of mitochondrial components from stimulated live mast cells may act as "autopathogens" contributing to the pathogenesis of inflammatory diseases and may be used as targets for novel treatments.

PMID: 23284625 **Free Article**
http://www.ncbi.nlm.nih.gov/pubmed/23284625

Cerebellum. 2012 Jun;11(2):575-86. doi: 10.1007/s12311-011-0319-5.

Maternal thimerosal exposure results in aberrant cerebellar oxidative stress, thyroid hormone metabolism, and motor behavior in rat pups; sex- and strain-dependent effects.

Sulkowski ZL, Chen T, Midha S, Zavacki AM, Sajdel-Sulkowska EM.

Department of Psychiatry, Harvard Medical School and Brigham and Women's Hospital, Boston, MA, USA.

Abstract

Methylmercury (Met-Hg) and ethylmercury (Et-Hg) are powerful toxicants with a range of harmful neurological effects in humans and animals. While Met-Hg is a recognized trigger of oxidative stress and an endocrine disruptor impacting neurodevelopment, the developmental neurotoxicity of Et-Hg, a metabolite of thimerosal (TM), has not been explored. We hypothesized that TM exposure during the perinatal period impairs central nervous system development, and specifically the cerebellum, by the mechanism involving oxidative stress. To test this, spontaneously hypertensive rats (SHR) or Sprague-Dawley (SD) rat dams were exposed to TM (200 μg/kg body weight) during pregnancy (G10-G15) and lactation (P5-P10). Male and female neonates were evaluated for auditory and motor function; cerebella were analyzed for oxidative stress and thyroid metabolism. TM exposure resulted in a delayed startle response in SD neonates and decreased motor learning in SHR male (22.6%), in SD male (29.8%), and in SD female (55.0%) neonates. TM exposure also resulted in a significant increase in cerebellar levels of the oxidative stress marker 3-nitrotyrosine in SHR female (35.1%) and SD male (14.0%) neonates. The activity of cerebellar type 2 deiodinase, responsible for local intra-brain conversion of thyroxine to the active hormone, 3',3,5-triiodothyronine (T3), was significantly decreased in TM-exposed SHR male (60.9%) pups. This coincided with an increased (47.0%) expression of a gene negatively regulated by T3, Odf4 suggesting local intracerebellar T3 deficiency. Our data thus demonstrate a negative neurodevelopmental impact of perinatal TM exposure which appears to be both strain- and sex-dependent.

PMID: 22015705
http://www.ncbi.nlm.nih.gov/pubmed/22015705

J Physiol Pharmacol. 2012 Jun;63(3):277-83.

Sex-dependent changes in cerebellar thyroid hormone-dependent gene expression following perinatal exposure to thimerosal in rats.

Khan A, Sulkowski ZL, Chen T, Zavacki AM, Sajdel-Sulkowska EM.

Department of Psychiatry, Harvard Medical School/BWH, Boston, MA 02115, USA.

Abstract

Mammalian brain development is regulated by the action of thyroid hormone (TH) on target genes. We have previously shown that the perinatal exposure to thimerosal (TM, metabolized to ethylmercury) exerts neurotoxic effects on the developing cerebellum and is associated with a decrease in cerebellar D2 activity, which could result in local brain T3 deficiency. We have also begun to examine TM effect on gene expression. The objective of this study was to expand on our initial observation of altered cerebellar gene expression following perinatal TM exposure and to examine additional genes that include both TH-dependent as well as other genes critical for cerebellar development in male and female neonates exposed perinatally (G10-G15 and P5 to P10) to TM. We report here for the first time that expression of suppressor-of-white-apricot-1 (SWAP-1), a gene negatively regulated by T3, was increased in TM-exposed males (61.1% increase), but not in females; ($p<0.05$). Positively regulated T3-target genes, Purkinje cell protein 2 (Pcp2; $p=0.07$) and Forkhead box protein P4 (FoxP4; $p=0.08$), showed a trend towards decreased expression in TM-exposed males. The expression of deiodinase 2 (DIO2) showed a trend towards an increase in TM-exposed females, while deiodinase 3 (DIO3), transthyretin (TTR), brain derived neurotrophic factor (BDNF) and reelin (RELN) was not significantly altered in either sex. Since regulation of gene splicing is vital to neuronal proliferation and differentiation, altered expression of SWAP-1 may exert wide ranging effects on multiple genes involved in the regulation of cerebellar development. We have previously identified activation of another TH-dependent gene, outer dense fiber of sperm tails 4, in the TM exposed male pups. Together, these results also show sex-dependent differences between the toxic impacts of TM in males and females. Interestingly, the genes that were activated by TM are negatively regulated by TH, supporting our hypothesis of local brain hypothyroidism being induced by TM and suggesting a novel mechanism of action TM in the developing brain.

PMID: 22791642 **Free Article**

http://www.ncbi.nlm.nih.gov/pubmed/22791642
http://www.jpp.krakow.pl/journal/archive/06_12/pdf/277_06_12_article.pdf

Autism Res Treat. 2012;2012:190930. doi: 10.1155/2012/190930. Epub 2012 Jun 19.

Prenatal and Postnatal Epigenetic Programming: Implications for GI, Immune, and Neuronal Function in Autism.

Waly MI, Hornig M, Trivedi M, Hodgson N, Kini R, Ohta A, Deth R.

Source

Department of Food Science and Nutrition, Sultan Qaboos University, Alkoudh 123, Muscat, Oman.

Abstract

Although autism is first and foremost a disorder of the central nervous system, comorbid dysfunction of the gastrointestinal (GI) and immune systems is common, suggesting that all three systems may be affected by common molecular mechanisms. Substantial systemic deficits in the antioxidant glutathione and its precursor, cysteine, have been documented in autism in association with oxidative stress and impaired methylation. DNA and histone methylation provide epigenetic regulation of gene expression during prenatal and postnatal development. Prenatal epigenetic programming (PrEP) can be affected by the maternal metabolic and nutritional environment, whereas postnatal epigenetic programming (PEP) importantly depends upon nutritional support provided through the GI tract. Cysteine absorption from the GI tract is a crucial determinant of antioxidant capacity, and systemic deficits of glutathione and cysteine in autism are likely to reflect impaired cysteine absorption. Excitatory amino acid transporter 3 (EAAT3) provides cysteine uptake for GI epithelial, neuronal, and immune cells, and its activity is decreased during oxidative stress. Based upon these observations, we propose that neurodevelopmental, GI, and immune aspects of autism each reflect manifestations of inadequate antioxidant capacity, secondary to impaired cysteine uptake by the GI tract. Genetic and environmental factors that adversely affect antioxidant capacity can disrupt PrEP and/or PEP, increasing vulnerability to autism.

PMID: 22934169 **Free Article**
http://www.ncbi.nlm.nih.gov/pubmed/22934169

PLoS One. 2013;8(2):e56927. doi: 10.1371/journal.pone.0056927. Epub 2013 Feb 20.

Age-dependent decrease and alternative splicing of methionine synthase mRNA in human cerebral cortex and an accelerated decrease in autism.

Muratore CR, Hodgson NW, Trivedi MS, Abdolmaleky HM, Persico AM, Lintas C, De La Monte S, Deth RC.

Department of Pharmaceutical Sciences, School of Pharmacy, Northeastern University, Boston, Massachusetts, United States of America.

Abstract

The folate and vitamin B12-dependent enzyme methionine synthase (MS) is highly sensitive to cellular oxidative status, and lower MS activity increases production of the antioxidant glutathione, while simultaneously decreasing more than 200 methylation reactions, broadly affecting metabolic activity. MS mRNA levels in postmortem human cortex from subjects across the lifespan were measured and a dramatic progressive biphasic decrease of more than 400-fold from 28 weeks of gestation to 84 years was observed. Further analysis revealed alternative splicing of MS mRNA, including deletion of folate-binding domain exons and age-dependent deletion of exons from the cap domain, which protects vitamin B12 (cobalamin) from oxidation. Although three species of MS were evident at the protein level, corresponding to full-length and alternatively spliced mRNA transcripts, decreasing mRNA levels across the lifespan were not associated with significant changes in MS protein or methionine levels. MS mRNA levels were significantly lower in autistic subjects, especially at younger ages, and this decrease was

replicated in cultured human neuronal cells by treatment with TNF-α, whose CSF levels are elevated in autism. These novel findings suggest that rather than serving as a housekeeping enzyme, MS has a broad and dynamic role in coordinating metabolism in the brain during development and aging. Factors adversely affecting MS activity, such as oxidative stress, can be a source of risk for neurological disorders across the lifespan via their impact on methylation reactions, including epigenetic regulation of gene expression.

PMID: 23437274 **Free Article**
http://www.ncbi.nlm.nih.gov/pubmed/23437274

J Neuroinflammation. 2013 Apr 9;10(1):46. [Epub ahead of print]

Focal brain inflammation and autism.

Theoharides TC, Asadi S, Patel AB.

Abstract

Increasing evidence indicates that brain inflammation is involved in the pathogenesis of neuropsychiatric diseases. Autism spectrum disorders (ASD) are characterized by social and learning disabilities that affect as many as 1/80 children in the USA. There is still no definitive pathogenesis or reliable biomarkers for ASD, thus significantly curtailing the development of effective therapies. Many children with ASD regress at about age 3 years, often after a specific event such as reaction to vaccination, infection, stress or trauma implying some epigenetic triggers, and may constitute a distinct phenotype. ASD children respond disproportionally to stress and are also affected by food and skin allergies. Corticotropin-releasing hormone (CRH) is secreted under stress and together with neurotensin (NT) stimulates mast cells and microglia resulting in focal brain inflammation and neurotoxicity. NT is significantly increased in serum of ASD children along with mitochondrial DNA. NT stimulates mast cell secretion of mitochondrial DNA that is misconstrued as an innate pathogen triggering an auto-inflammatory response. Lack of the mammalian target of rapamycin (mTOR), which is inhibited by the phosphatase and tensin homolog (PTEN), has been linked to gene mutations associated with higher risk of ASD. CRH, NT and environmental triggers could hyperstimulate the already activated mTOR leading to higher risk for ASD, as well as stimulate mast cell and microglia activation and proliferation. The natural flavonoid luteolin inhibits mTOR, mast cells and microglia and could have a significant benefit in ASD.

PMID: 23570274 **Free Article**
http://www.jneuroinflammation.com/content/10/1/46
http://www.ncbi.nlm.nih.gov/pubmed/23570274