

Neurodevelopmental Disorders, Maternal Rh-Negativity, and Rho(D) Immune Globulins: A Multi-Center Assessment

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Abstract

BACKGROUND: Many formulations of Thimerosal (49.55% mercury by weight)-containing Rho(D) immune globulins (TCRs) were routinely administered to Rh-negative mothers in the US prior to 2002.

OBJECTIVES: It was hypothesized: (1) if prenatal Rho(D)-immune globulin preparation exposure was a risk factor for neurodevelopmental disorders (NDs) then more children with NDs would have Rh-negative mothers compared to controls; and (2) if Thimerosal in the Rho(D)-immune globulin preparations was the ingredient associated with NDs, following the removal of Thimerosal from all manufactured Rho(D)-immune globulin preparations from 2002 in the US the frequency of maternal Rh-negativity among children with NDs should be similar to control populations.

METHODS: Maternal Rh-negativity was assessed at two sites (Clinic A-Lynchburg, VA; Clinic B-Rockville and Baltimore, MD) among 298 Caucasian children with NDs and known Rh-status. As controls, maternal Rh-negativity frequency was determined from 124 Caucasian children (born 1987-2001) without NDs at Clinic A, and the Rh-negativity frequency was determined from 1,021 Caucasian pregnant mothers that presented for prenatal genetic care at Clinic B (1980-1989). Additionally, 22 Caucasian patients with NDs born from 2002 onwards (Clinics A and B) were assessed for maternal Rh-negativity.

RESULTS: There were significant and comparable increases in maternal Rh-negativity among children with NDs (Clinic: A=24.2%), autism spectrum disorders (Clinic: A=28.3%, B=25.3%), and attention-deficit-disorder/attention-deficit-hyperactivity-disorder (Clinic: A=26.3%) observed at both clinics in comparison to both control groups (Clinic: A=12.1%, B=13.9%) employed. Children with NDs born post-2001 had a maternal Rh-negativity frequency (13.6%) similar to controls.

CONCLUSION: This study associates TCR exposure with some NDs in children.

Potential Conflict of Interest: David Geier has been a consultant in legal cases involving vaccines/biologics. Dr. Elizabeth Mumper and Dr. Mark Geier have been expert witnesses and consultants in legal cases involving vaccines/biologics.

ABBREVIATIONS

ACOG	– American College of Obstetricians and Gynecologists
ADD	– attention-deficit-disorder
ADHD	– attention-deficit-hyperactivity-disorder
ASDs	– autistic spectrum disorders
MD	– Maryland
µg	– microgram
µM	– micromolar
nM	– nanomolar
NDs	– Neurodevelopmental Disorders
ppm	– part-per-million
TCRs	– Thimerosal (49.55% mercury by weight)-containing
Rho(D)	– immune globulins
VA	– Virginia

INTRODUCTION

Rho(D)-immune globulin is an immune globulin preparation containing antibodies to Rho(D) which is intended for intramuscular injection. Until 2001, when the last doses of Thimerosal-containing Rho(D)-immune globulin preparations were manufactured, many formulations of Rho(D)-immune globulin contained Thimerosal in the United States. Thimerosal is an ethylmercury-containing compound (49.55% mercury by weight) that was added to Rho(D)-immune globulin preparations at the preservative level of 0.003% to 0.01% (from 10.5 microgram [µg] mercury to > 35 µg mercury / dose) (Geier *et al.* 2007). Thimerosal dissociates in saline-based formulations into ethylmercuric chloride and thiosalicylic acid (Reader & Lines, 1983).

Rho(D)-immune globulin is used to prevent isoimmunization in the Rho(D) negative individual exposed to Rho(D) positive blood as a result of fetomaternal hemorrhage occurring during delivery of an Rho(D) positive infant, abortion (either spontaneous or induced), or following amniocentesis or abdominal trauma. Rh hemolytic disease of the newborn is the result of the active immunization of an Rho(D) negative mother by Rho(D) positive red cells entering maternal circulation during a previous delivery, abortion, amniocentesis, abdominal trauma, or as a result of red cell transfusion (American College of Obstetricians and Gynecologists, 1976; 1981). Rho(D)-immune globulin acts by suppressing the immune response of Rho(D) negative individuals to Rho(D) positive red blood cells. The mechanism of action of Rho(D)-immune globulin is not fully understood.

Historically, Rho(D)-immune globulin was administered within 72 hours of a full-term delivery of a Rho(D) positive infant by a Rho(D) negative mother or following known potential exposure between maternal and fetal blood. It was observed that administration of Rho(D)-immune globulin under such guidelines reduced the incidence of Rh isoimmunization from 12% to 13% to 1%-2% (Pollack, 1978). It was reported that the 1%-2% of treatment failures that continued to occur probably resulted from isoimmunization occurring during the latter part of pregnancy or following pregnancy (Bow-

man *et al.*, 1978). Bowman and Pollock (1978) showed that the incidence of isoimmunization could be further reduced from approximately 1.6% to less than 0.1% by administering Rho(D)-immune globulin preparations in two doses, one antenatal at 28 weeks' gestation and another following delivery. As a result, in the late 1980s/early 1990s, the American College of Obstetricians and Gynecologists (ACOG) adopted the recommendation that in addition to birth and times of potential mixing of fetal and maternal blood, Rho(D)-immune globulin preparations should be routinely administered to all Rh-negative mothers at 28 weeks' gestation (American College of Obstetricians and Gynecologists, 1990).

This study focuses on maternal Rh-negativity and neurodevelopmental disorders (NDs). It was hypothesized that if prenatal Rho(D)-immune globulin preparation exposure was a risk factor for NDs then more children with NDs would have Rh-negative mothers compared to controls. Additionally, if Thimerosal in the Rho(D)-immune globulin preparations was the ingredient associated with NDs, it was also hypothesized that following the removal of Thimerosal from all manufactured Rho(D)-immune globulin preparations from 2002 in the US the frequency of maternal Rh-negativity among children with NDs should be similar to control populations.

MATERIAL AND METHODS**Subjects**

The frequency of maternal Rh-negativity was assessed among Caucasian children born from 1987 through 2001 at two separate clinics. At Clinic A (Advocates for Children Pediatrics Ltd., Lynchburg, Virginia [VA]) a total of 196 patients with NDs (including 88 patients diagnosed with autistic spectrum disorders (ASDs) and 95 patients diagnosed with attention-deficit-disorder (ADD) / attention-deficit-hyperactivity-disorder (ADHD)) were evaluated (patients examined could have more than one diagnosis). At Clinic B (The Genetic Centers of America, Rockville, Maryland [MD] and Baltimore, MD) 87 ASD patients were examined. Table 1 summarizes the overall profile of the children with NDs examined in the present study.

Evaluation

A review of each patient's medical records was undertaken to determine the patient's race and maternal Rh-status.

Controls

In order to evaluate the frequency of Rh-negativity in patients without NDs, the maternal Rh-status of 124 Caucasian patients born from 1987 through 2001 without NDs that presented for pediatric care were assessed at Clinic A (Control Group 1). The Rh-status of 1,021 Caucasian pregnant women that presented for outpatient prenatal genetic care from 1980 through 1989 were

Table 1. Study group profile of the patients examined in the present study

Group Type (n)	Number of males / females (ratio)	Median year of birth (range)	Maternal Rh-negative Percent (n)
Clinic A			
Neurodevelopmental Disorders ^a (194)	148 / 46 (3.2:1)	1996 (1987–2001)	24.2% (47)
Autism Spectrum Disorders ^b (92)	80 / 12 (6.7:1)	1997 (1987–2001)	28.3% (26)
Attention Deficit Disorder / Attention Deficit Hyperactivity Disorder (95)	64 / 31 (2.07:1)	1994 (1988–2000)	26.3% (25)
Control Group 1 ^c (124)	54 / 70 (0.77:1)	1998 (1987–2001)	12.1% (15)
Clinic B			
Autism Spectrum Disorders ^b (87)	76 / 11 (6.9:1)	1997 (1987–2001)	25.3% (22)
Control Group 2 ^d (1,021)	–	–	13.9% (142)
Clinics A & B			
Control Group 3 ^e (22)	18 / 4 (4.5:1)	2002 (2002–2004)	13.6% (3)

^a Includes patients diagnosed with pervasive developmental delay-not otherwise specified, Asperger's disorder, autism, attention deficit disorder, attention deficit hyperactivity disorder, speech delay/language delay, cerebral palsy, anxiety disorder, obsessive compulsive disorder, sleep disorder, apraxia, motor delay, tics, or developmental delay

^b Includes patients diagnosed with autism, pervasive developmental delay-not otherwise specified or Asperger's disorder (patients could have more than one diagnosis).

^c Caucasian children not diagnosed with a neurodevelopmental disorder.

^d Caucasian pregnant women that presented for prenatal genetic care from 1980 through 1989.

^e Caucasian patients with neurodevelopmental disorders from Clinics A and B born from 2002 onwards.

assessed at Clinic B (Control Group 2). These frequencies were determined by reviewing each of the patient's medical records. Additionally, the frequency of maternal Rh-negativity was assessed among 22 patients with NDs at Clinics A and B born after 2001 (Control Group 3). Table 1 summarizes the overall profile of the controls examined in the present study.

Statistical Analyses

In the present study, the statistical package contained in StatsDirect™ (Version 2.4.2) was employed. The null hypothesis was that the maternal frequency of Rh-negativity would be similar among those with or without NDs. The Fisher's exact test statistic was utilized to determine statistical significance. A two-tailed *p* value ≤ 0.05 was considered statistically significant.

RESULTS

Table 2 summarizes the frequency of maternal Rh-negativity in children with NDs at Clinics A and B in comparison to controls. It was observed that the frequency of maternal Rh-negativity was significantly increased at Clinic A among patients with NDs, ADD/ADHD, and ASDs in comparison to Control Groups 1 and 2. Additionally, it was observed that the frequency of maternal Rh-negativity was significantly increased at Clinic B among patients with ASDs in comparison to Control

Groups 1 and 2. Finally, it was observed that the frequency of maternal Rh-negativity among patients with NDs born after 2001 (Control Group 3) was similar to the frequency of maternal Rh-negativity observed among the other control populations examined (Control Groups 1 and 2).

DISCUSSION

In the present study, an examination of the relationship between maternal Rh-negativity, Rho(D)-immune globulins, and NDs was undertaken. It was observed that Caucasian children examined with NDs born from 1987 through 2001 were significantly more likely to have Rh-negative mothers than Caucasian children without NDs born from 1987 through 2001 that presented for outpatient pediatric care or among a series of Caucasian mothers that presented for outpatient prenatal genetics care from 1980 through 1989. It was also observed that Rh-negativity among Caucasian children with NDs born after 2001 had a similar frequency of Rh-negative mothers as controls.

Study Design Considerations

In considering the patients evaluated in the present study, Caucasian patients with NDs that prospectively presented to Clinic A for outpatient pediatric care and to Clinic B for outpatient genetics care were examined.

The maternal Rh-status of the patients was not a selection criterion for patient presenting for their evaluations at Clinics A and B.

The children examined with NDs in the present study were matched to controls based upon race. At Clinic A information regarding race and maternal Rh-negativity was derived by a retrospective review of all medical records of children without NDs that previously presented for outpatient pediatric care. At Clinic B maternal Rh-negativity that was derived from pregnant women for outpatient prenatal genetics care, and hence it was of integral importance to the management of each patient's pregnancy to determine their Rh-status and their racial demographic information. As a result, the method of analysis employed attempted to ensure maximum capture of Rh-status, and attempt to control for potential racial differences in the rate of Rh-negativity.

The diagnoses for the children examined with NDs in the present study were derived based upon retrospective chart review. At Clinic A information was collected regarding various possible ND diagnoses and the patients were classified into three major categories including NDs, ADHD/ADD, or ASDs. The categories employed allowed for patients examined to have potentially more than one diagnosis. A review of the data in each category by only including those patients with a single diagnosis revealed minimal differences in the frequencies of maternal Rh-negativity observed in Table 1. By contrast, at Clinic B the entire population examined was diagnosed with ASDs.

In the present study attempts were made to minimize chance statistically significant associations. First, a two-tailed p -value ≤ 0.05 was selected to be statistically significant. As a result, by chance alone one in 20 outcomes would be expected to be statistically significant. Since, in the present study, a total of 11 statistical tests were conducted less than one of the results in the present study would be expected to be due to statistical chance. Furthermore, in all but one of the statistically significant results, the p -value was < 0.01 , and hence this further minimizes the potential that the results of the present study were due to chance. Second, the ND outcomes that were selected in the present study are ones that are biologically plausibly linked to mercury exposure, and hence were not selected based upon screening through multiple different outcome measures. Third, the consistency of the results obtained in the present study from prospectively collected data across the different outcome measures, control groups, and clinic sites examined, argues against the observations being due to mere chance or an unknown confounder.

The strength of the present study stems from the fact that children with NDs and controls were prospectively collected from two different clinics in two different geographical locations, and yet both yielded consistent significant associations between maternal Rh-negativity and NDs. A further strength of the present study is that

Table 2. A summary of the frequency of maternal Rh negativity among the children with neurodevelopmental disorders in comparison to controls.

Group Type (n)	Maternal Rh-negative Percent (n)
Clinic A	
Neurodevelopmental Disorders ^a (194)	24.2% (47)
Control Group 1 ^b (124)	12.1% (15)
Odds Ratio (95% CI) ^c	2.3 (1.2-4.4)
p -value	< 0.01
Control Group 2 ^d (1,021)	13.9% (142)
Odds Ratio (95% CI)	2.02 (1.4-3.0)
p -value	< 0.001
Autism Spectrum Disorders ^e (92)	28.3% (26)
Control Group 1 ^b (124)	12.1% (15)
Odds Ratio (95% CI) ^c	2.9 (1.3-6.2)
p -value	< 0.01
Control Group 2 ^d (1,021)	13.9% (142)
Odds Ratio (95% CI)	2.5 (1.5-4.1)
p -value	< 0.001
Attention Deficit Disorder / Attention Deficit Hyperactivity Disorder (95)	26.3% (25)
Control Group 1 ^b (124)	12.1% (15)
Odds Ratio (95% CI) ^c	2.6 (1.2-5.7)
p -value	< 0.01
Control Group 2 ^d (1,021)	13.9% (142)
Odds Ratio (95% CI)	2.3 (1.3-3.8)
p -value	< 0.01
Clinic B	
Autism Spectrum Disorders ^e (87)	25.3% (22)
Control Group 1 ^b (124)	12.1% (15)
Odds Ratio (95% CI) ^c	2.5 (1.1-5.5)
p -value	< 0.05
Control Group 2 ^d (1,021)	13.9% (142)
Odds Ratio (95% CI)	2.1 (1.2-3.6)
p -value	< 0.01

CI = Confidence Interval

The frequency of Rh-negativity between Control Group 1 and Control Group 2 were not significantly different (odds ratio = 1.2, 95% CI = 0.66-2.2)

^a Includes patients diagnosed with pervasive developmental delay-not otherwise specified, Asperger's disorder, autism, attention deficit disorder, attention deficit hyperactivity disorder, speech delay, language delay, cerebral palsy, anxiety disorder, obsessive compulsive disorder, sleep disorder, apraxia, motor delay, tics, or developmental delay (patients could have more than one diagnosis).

^b Caucasian children not diagnosed with a neurodevelopmental disorder.

^c The Fisher's Exact test statistic (two-tailed p value) was employed to determine statistical significance.

^d Caucasian pregnant women that presented for obstetrical care from 1980 through 1989.

^e Includes patients diagnosed with autism, pervasive developmental delay-not otherwise specified or Asperger's disorder.

for children with NDs born after 2001, when only Thimerosal-free Rho(D) immune globulin preparations were manufactured for the US market, the frequency of maternal Rh-negativity among children with NDs returned to the frequency observed in controls. It is important to note that the only significant constituent component removed from Rho(D) immune globulin preparations post-2001 was Thimerosal.

The main limitation of the present study was that individual exposure levels to mercury from Thimerosal-containing Rho(D) immune globulins could not be assessed. This information could provide further information on the relationship between Thimerosal and NDs, since different manufacturers of Rho(D) immune globulins at different times had different concentrations of Thimerosal. This is an area of study that deserves further examination, but if anything such effects, if present, would have tended to bias the results towards the null hypothesis in the present study. Furthermore, mercury burden from other sources, such as thimerosal containing pediatric vaccines, inhaled environmental exposures, maternal amalgams, maternal fish consumption or ingested methylmercury, was not calculated. Additionally, only a limited number of children with NDs born after 2001 were assessed in the present study, but further data can be collected to see if the present trend is confirmed.

The Caucasian controls maternal rate of Rh-negativity observed in the present study of 12.1% at Clinic A and 13.9% at Clinic B is consistent with rates observed by researchers in several other populations including Lurie *et al.* (2003) (8.6%), Holmes *et al.* (2003) (9%), Geier and Geier (2007) (14.36%), and Garratty *et al.* (2004) (17.3%). The estimate by Garratty *et al.* (2004) may be skewed because it was derived from a blood bank where oftentimes specific types of blood are requested, especially Rh-negative blood types. Additionally, the maternal frequency of Rh-negativity observed in the present study among those with NDs born from 1987 through 2001 at Clinic A or Clinic B were observed to be significantly elevated in comparison with these other populations.

Holmes *et al.* (2003) observed that the mothers of autistic children had a significantly increased frequency of maternal Rh-negativity and more injections of Rho(D)-immune globulin preparations administered than controls. Similarly, it was also reported in another study that children with ASDs were significantly more likely to have Rh-negative mothers than controls, and each ASD patient's mother was determined to be administered at least one Thimerosal-containing Rho(D)-immune globulin preparation during her pregnancy (Geier & Geier, 2007c). Other researchers have associated Thimerosal-containing drugs administered during pregnancy or early postnatally with NDs (Lathe, 2006; 2008; Maya and Luna, 2006; Mutter *et al.* 2005; 2007).

By contrast, Miles and Takahashi (2007) evaluated and reported that there was no indication that pregnancies of children with autism were any more likely to have received Rho(D)-immune globulins or have Rh-negative mothers than those of controls, but the analyses conducted had significant limitations that have recently been described (Bernard *et al.* 2008). The following are a few specific limitations including: (1) the absence of a neurotypical children control group to evaluate the general population frequency of maternal Rh-negativity and Rho(D)-immune globulin administration (controls included blood bank estimates of the frequency of Rh-negativity and children with chromosomal abnormalities); (2) > 50% of families with children diagnosed with autistic disorders were lost to follow-up according to the authors; and (3) information on the racial demographics on several of the control groups employed was not provided. Furthermore, it is difficult to reconcile the results observed by Miles and Takahashi (2007) with their previous data that identified in their population of autistic patients from several different ethnic backgrounds a rate of maternal Rh-negativity = 19.5% (Miles & Takahashi, 2005).

Biological Plausibility

Faustman *et al.* (2000) reported, "...mercury exposure altered cell number and cell division; these impacts have been postulated as modes of action for the observed adverse effects in neuronal development. The potential implications of such observations are evident when evaluated in context with research showing that altered cell proliferation and focal neuropathologic effects have been linked with specific neurobehavioral deficits (e.g., autism)." Additionally, Zahir *et al.* (2005) described that the access of mercury, "...to man through multiple pathways air, water, food, cosmetic products and even vaccines increase the exposure. Fetuses and children are more susceptible towards mercury toxicity. Mothers consuming diet containing mercury pass the toxicant to fetus and to infants through breast milk. Decreased performance in the areas of motor function and memory has been reported among children exposed to presumably safe mercury levels...Mercury has been found to be a causative agent of various sorts of disorders, including neurological, nephrological, immunological, cardiac, motor, reproductive and even genetic. Recently heavy metal mediated toxicity has been linked to diseases like Alzheimer's, Parkinson's, Autism, Lupus, Amyotrophic lateral sclerosis, etc."

Additionally, it was observed in previous epidemiological studies that there was a significant association between low-dose mercury exposure and NDs (Amin-Zaki *et al.* 1981; Counter *et al.* 2002; Debes *et al.* 2006; Geier & Geier 2006a; Jedrychowski *et al.* 2006; Marques *et al.* in press; Palmer *et al.* 2006; in press; Rury, 2006; Windham *et al.* 2006; Young *et al.* in press). Furthermore, it was observed in mercury poisonings in humans that prenatal exposure to organic mercury can

result in signs/symptoms of developmental toxicity that begin to manifest more than six months following birth (Harada, 1978).

Thimerosal has been recognized by the California Environmental Protection Agency, Office of Environmental Health Hazard Assessment as a developmental toxin. This implies that Thimerosal may produce birth defects, low birth weight, biological dysfunctions, or psychological or behavior deficits that become manifest as the child grows. Maternal exposure during pregnancy may disrupt the development or even cause the death of the fetus.

It was shown that administration of prenatal Thimerosal to animals at less than 1 part-per-million (ppm) can induce significant fetal lethality and teratogenicity in a dose-dependent fashion (Digar *et al.* 1987; Gasset *et al.* 1975; Itoi *et al.* 1972). Heinonen *et al.* (1977) examined 2,277 children with birth defects among 50,282 mother-child pairs and determined that Thimerosal exposure during the first 4 months of pregnancy was associated with a significantly increased risk (survival and race standardized relative risk = 2.69) for birth defects. Similar phenomena were observed in human poisonings with ethylmercury (Bakulina, 1968; Mal'tsev, 1972; Ramanauskayte & Baublis, 1973). Bakulina (1968) described in human exposure, "...ethylmercury chloride is capable of passing through the placental barrier and penetrating into the fetus, causing in the organs of the latter grave pathological changes. The permeability of the placental barrier for organic mercury compounds finds its confirmation in the presence of mercury in the placenta and organs of the fetus..."

Hornig *et al.* (2004) administered Thimerosal to mice at doses and ages mimicking the childhood immunization schedule of the 1990s, and observed symptoms of NDs in a susceptible mouse strain characterized by autoimmunity. The Thimerosal exposed mice exhibited growth delay, reduced locomotion, exaggerated response to novelty, increased brain size, decreased numbers of Purkinje cells, significant abnormalities in brain architecture affecting areas subserving emotion and cognition, and densely packed hyperchromic hippocampal neurons with altered glutamate receptors and transporters. By contrast, Berman *et al.* (2008) conducted a study to evaluate the effects of injected Thimerosal in a neonatal mouse strain. These researchers concluded that considered together their results did not indicate pervasive developmental neurotoxicity following vaccine-level Thimerosal injections in mice, and provide little if any support for the hypothesis that Thimerosal exposure contributes to the etiology of neurodevelopmental disorders.

A series of molecular studies with neurons demonstrated that nanomolar (nM) to micromolar (μ M) concentrations of Thimerosal are capable of inducing pathological changes observed in the brains of patients

diagnosed with NDs including neuronal death, neurodegeneration, membrane damage, and DNA damage (Baskin *et al.* 2003; Brown & Yel, 2003; Brunner *et al.* 1991; Haley, 2005; Herdman *et al.*, 2006; Humphrey *et al.* 2005; James *et al.* 2005; Wallin & Hartely-Asp, 1993; Yel *et al.* 2005). Additionally, it has also been shown that nM to μ M concentrations of Thimerosal are capable of disrupting critical signaling pathways/biochemical events necessary for neurons to undergo normal neuronal development (Mutkus *et al.*, 2005; Parran *et al.*, 2005; Waly *et al.* 2004).

The mercury kinetics of prenatal/postnatal Thimerosal administration show that the ethylmercury from Thimerosal by various routes of administration is capable of crossing the placental and blood-brain barriers and results in appreciable persistent bound inorganic mercury content in tissues including the brain (Blair *et al.*, 1975; Burbacher *et al.* 2005; Fagan *et al.* 1977; Gasset *et al.* 1975; Leonard *et al.* 1983; Slikker, 2000; Suzuki *et al.* 1973). It was also shown that exposure to ethylmercury results in a greater mercury concentration in fetal tissues than the mother, especially in the fetal central nervous system (Ukita *et al.* 1967).

In examining the retention of mercury in tissues following injection of Thimerosal into infant monkeys, it was shown that the half-life for organic mercury in the brain was about 14 days (Burbacher *et al.* 2005). Furthermore, it was observed that there was a significant inorganic mercury concentration in the brain following injection of Thimerosal into infant monkeys and the half-life for the inorganic mercury in the brain was too long to estimate a value from the available data; no significant measurable decline was detectable by 120 days (Burbacher *et al.* 2005).

The overall importance of persistent inorganic mercury in the brain stems from the fact that a number of recent studies showed that dealkylation of mercury in the brain is not a detoxification process (Charleston *et al.* 1994; 1995; 1996; Vahter *et al.* 1994; 1995). Following dosing with organic mercury that the half-life of inorganic mercury in the brain was estimated to vary significantly across different regions of the brain, from 227 days to 540 days. In other regions, the concentrations of inorganic mercury remained the same (in the thalamus) or doubled (in the pituitary) six months after mercury dosing had ended (Vahter *et al.* 1994; 1995). Stereologic and autometallographic studies on the brains of monkeys indicated that the persistence of inorganic mercury in the brain was associated with a significant increase in the number of microglia in the brain, whereas the number of astrocytes declined. Notably, these effects were observed 6 months after mercury dosing had ended, when inorganic mercury concentrations were at their highest levels, or in animals solely exposed to inorganic mercury (Charleston *et al.*, 1994; 1995; 1996). These observations are important because "an active neuroinflammatory process" including a marked activation of

microglia was shown in pathological examinations of the brains of some with NDs (Vargas *et al.* 2005).

Clinical Studies

A series of recent clinical studies on patients diagnosed with NDs has revealed significant elevations in mercury concentrations. For example, Cheuk and Wong (2006) in patients diagnosed with attention-deficit hyperactivity disorder and Desoto and Hitlan (2007) in patients diagnosed with autistic disorders, both found significant elevations in blood mercury levels in comparison with controls. Adams *et al.* (2007) observed significant increases in the mercury levels of baby teeth in patients with autistic disorders in comparison with controls. Others have observed significant elevations in biomarkers associated with mercury toxicity among several cohorts of patients diagnosed with autistic disorders (Nataf *et al.* 2006; Geier & Geier 2006b; 2007a-b). Finally, several recent brain pathology studies have revealed elevations in mercury concentrations and mercury-associated oxidative stress markers in patients diagnosed with autistic disorders in comparison with controls (Evans *et al.* 2008; Lopez-Hurtado & Prieto, 2008; Sajdel-Sulkowska *et al.* 2008).

CONCLUSION

It is clear from these data that additional ND research should be undertaken in the context of evaluating mercury-associated exposures, especially from Thimerosal-containing Rho(D)-immune globulins administered during pregnancy. Further studies should also be undertaken in additional databases/registries to assess the compatibility of the present results with trends in NDs in other US populations, and to observe whether Thimerosal-containing Rho(D)-immune globulins were associated with other birth defects in children.

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