Original Paper

Journal of Biomedical Science

J Biomed Sci 2004;11:607–610
DOI: 10.1159/000079672

Received: March 16, 2004
Accepted: April 5, 2004

Detection of Antinuclear and Antilaminin Antibodies in Autistic Children Who Received Thimerosal-Containing Vaccines

Vijendra K. Singh Wyatt H. Rivas

Department of Biology, Utah State University, Logan, Utah, USA

Key Words
Autism · Autoimmunity · Immunotoxicity · Mercury · Thimerosal

Abstract
Autism, a neurodevelopmental disorder, may involve autoimmune pathogenesis. Since mercury is potentially a risk factor for autoimmunity, we conducted a study of mercury-induced antinuclear and antilaminin antibodies in autistic and normal children who had been pre-administered with thimerosal-containing vaccines. Laboratory analysis by different immunoassays showed that the serum level of these two autoimmune markers did not significantly differ between autistic and normal children. This finding suggests that the mercury as in thimerosal-containing vaccines is likely not related to autoimmune phenomenon in autism.

Introduction

Autism is an early-onset developmental disability that causes neurological deficits in children, affecting their ability for social interaction, language, imagination, communication and cognition. The etiopathogenesis of autism is not well established but it is generally considered as a multifactorial disorder. Current theories include the environmental, genetic, immune, neurochemical, developmental psychology, and other unknown factors. As recently reviewed [8], immune factors such as autoimmunity might play a pathogenic role in autism. Based on theoretical considerations of certain common behavioral manifestations in subjects with mercury exposure and autistic disorder, the exposure to mercury has recently been speculated as a risk factor for autism [1]. In contrast, however, the literature review by researchers did not find this commonality of behavioral manifestations [5]. Furthermore, the laboratory analysis showed that the blood levels of mercury do not rise above safe levels in infants and children receiving thimerosal-containing vaccines [6]. These observations prompted us to examine the possibility of a potential relationship between mercury exposure and autoimmune response in autism. We hypothesized that if autism involved such a connection then autistic children should harbor elevated levels of mercury-induced autoimmune markers, in particular the antinuclear antibodies (ANA) against nucleolar antigens and the antilaminin antibodies against basement-membrane-derived laminin protein [2, 4]. We therefore profiled these two autoimmune markers in autistic and normal children. The results of the present study show that the distribution of these two markers did not change in autistic children, thus the exposure to vaccine-derived mercury may not be related to autoimmune response in autism.

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1021–7770/04/0115–0607$21.00/0
Accessible online at:
www.karger.com/jbs

Vijendra Singh, PhD
Biotechnology Center Building, Utah State University
UMC 4700, Logan, UT 84322 (USA)
Tel. +1 435 797 7193, Fax +1 435 797 2766
E-Mail singhvk@cc.usu.edu

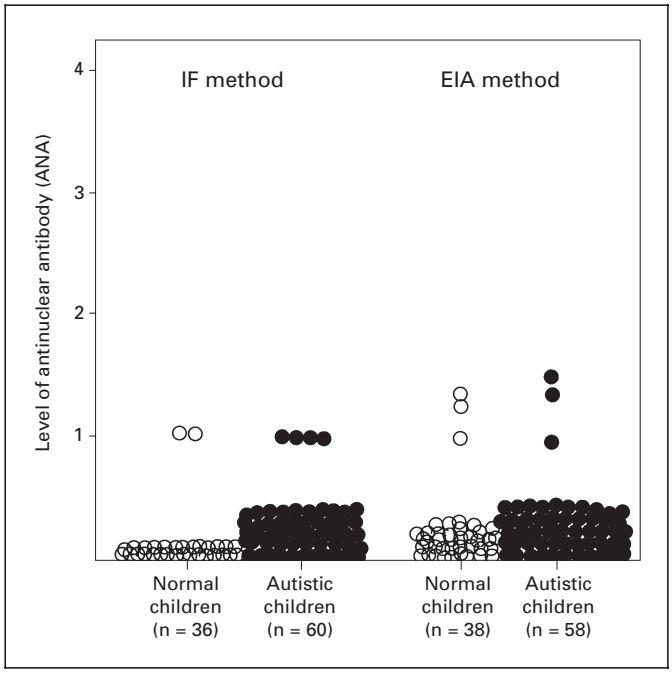

Fig. 1. Distribution of ANA in autistic and normal children. Sera from autistic and normal children were tested by the IF and EIA methods at a screening serum dilution of 1:40 as recommended by the manufacturers of the IF and EIA kits. The ANA level was scored on a scale of 0–4 either as the fluorescence staining intensity for the IF method or as the ANA index for the EIA method. As shown, >93% of normal and autistic sera were negative below the cutoff level of 1, and the remainder (7%) of sera showed a weak or low positive reaction slightly above the cutoff level of 1.

Patients and Methods

The study included 60 autistic children (aged 6.7 ± 3.8 years), 46 normal children (aged 7.4 ± 3.8 years) and 9 normal siblings (aged 5.8 ± 2.9 years) of the autistic children. However, due to limited resources, not all subjects were tested for all markers. As described elsewhere [8–10], the clinical diagnosis of autism and pervasive developmental disorders was essentially according to the standard DSM-IV criteria of the American Association of Psychiatrists, Washington, D.C., USA. The Institutional Review Board of the Utah State University reviewed and approved our research protocol that involved the use of human blood samples. All children were without any prescription medications at the time of blood collection. As required by the Immunization Program of the US Centers for Disease Control and Prevention, all children had received their full immunization schedule with multiple vaccines, including diphtheria-tetanus-pertussis, hepatitis B, inactivated polio vaccine, *Haemophilus influenzae* type b, and measles-mumps-rubella. Therefore, as far as we know, the children in the study were exposed to mercury from thimerosal-containing vaccines only but not to any other source of the mercury. In the present study, we used previously [8–10] collected serum samples that were stored frozen at –20°C. ANA were assayed by two different methods, namely the immunofluorescent (IF) method and the enzyme immunoassay (EIA) method using commercially available kits (ANA-IF kits from Sigma Diagnostics, St. Louis, Mo., and ANA-EIA kits from Biomeda, Foster City, Calif., USA). All procedures including the interpretation of the results were used in strict compliance with the manufacturer's instructions. For the IF method, the fluorescent staining pattern was scored between 0 and 4+: <1 being negative (cutoff level), 1+ being weakly positive, and 4+ being strongly positive. For the EIA method, the ANA index (AI), defined as the optical density ratio between a sample and the end-point control, was scored between 1 and 4+: the AI <1 being negative (cutoff level), AI >1 being low positive, and AI >2 being positive. The assay method for antilaminin antibodies was an EIA using pure laminin protein (ICN Biomedicals, Aurora, Ohio, USA) as the screening antigen. Briefly, this method was essentially the same as our recently published ELISA method [10] except that the microplates were coated with 2.5 μg laminin/well, and the goat anti-human-IgG-alkaline phosphatase was diluted to 1:800 in the second antibody step. The 0.1 optical density unit was arbitrarily defined as 10 EIA units. The data were evaluated by Student's t test.

Results and Discussion

The results of the laboratory analysis of ANA by two different methods are shown in figure 1. This marker was found to be present in both populations but notably in a very small percentage of children: 6–8% of the normal children and 5–7% of the autistic children were positive. However, the difference between these two groups was not significant ($p > 0.8$ for the IF method and $p > 0.5$ for the EIA method). The sera positive by the IF method showed fluorescent staining intensity of only 1+ and the sera positive by the EIA method had an ANA index in the low range (1.0–1.6). The positive samples showed a homogeneous ANA pattern, which is not characteristic of nucleolar antigens. Moreover, the ANA reaction was not blocked by pre-incubation with monoclonal antibody to nucleolar antigen (Biomeda, Foster City, Calif., USA; data were not included in this study). This finding suggests that the antibody reaction, although found in only 3 or 4 cases, was not due to ANA. Thus, the autistic children did not harbor ANA.

The measurement of antilaminin antibodies was carried out first at four serum dilutions: 1:20, 1:40, 1:80 and 1:160 dilutions of 6 autistic sera and 6 normal sera. As shown in figure 2, the level of antilaminin antibodies at these four serum dilutions did not differ significantly (p values being ≥0.385 for all dilutions) between autistic children and normal children. Since the serum dilution of 1:20 yielded the highest detectable level of antibody reaction, it was subsequently used to screen all other sera. The level of antilaminin antibodies in autistic children did not

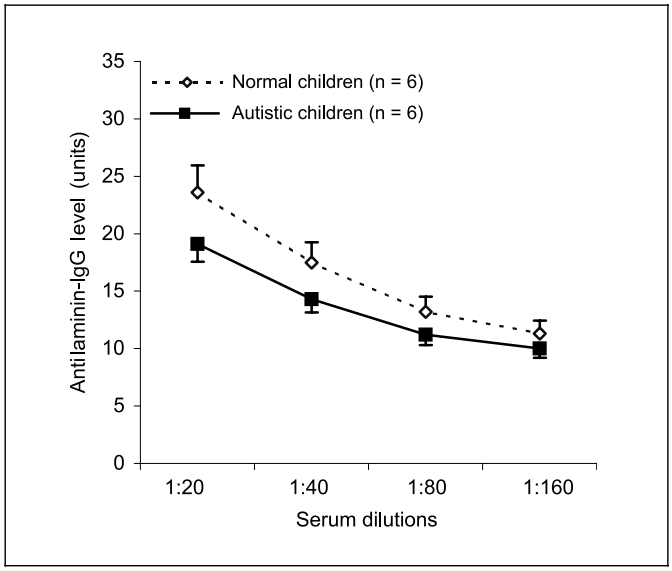

Fig. 2. Effect of serum dilution on antilaminin antibodies in autistic children. Sera from autistic and normal children were appropriately diluted, followed by the detection of antilaminin antibodies. The data given as the arithmetic means ± SE did not significantly differ between the patient and normal groups (p values being 0.385, 0.506, 0.397 and 0.424 at 1:20, 1:40, 1:80 and 1:160 dilutions, respectively).

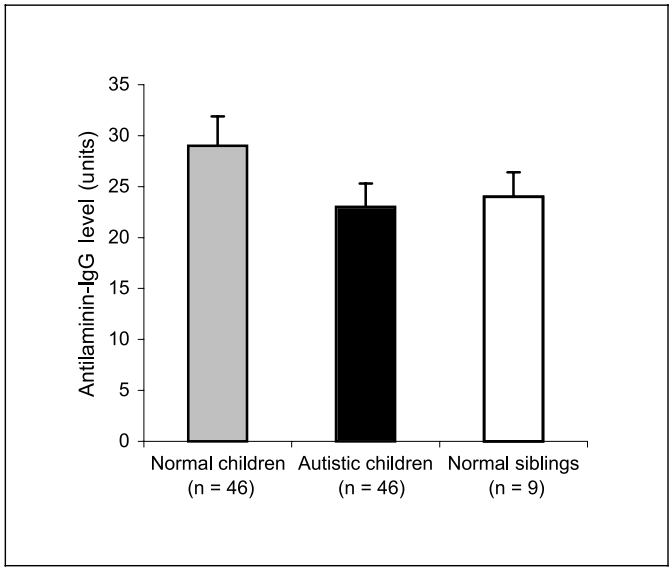

Fig. 3. Distribution of antilaminin antibodies in autistic children. All sera were pre-diluted to a 1:20 dilution, followed by the screening of antilaminin antibodies. The three subject groups were: normal children without any symptomatic illness (grey bar), autistic children with a diagnosis of autism (black bar), and normal siblings of autistic children (white bar). There was no significant difference between autistic children and normal children (p values being ≥ 0.1).

significantly change (p values being ≥ 0.1) when compared to normal children or normal siblings of the autistic children (fig. 3). Thus there was no significant difference in the distribution of antilaminin antibodies in the autistic group and the normal groups.

Mercury is widespread in the environment. Humans are exposed to mercury mainly through consumption of contaminated fish and medicinal products such as vaccines, dental amalgams and skin ointments. Workers in factories and industries are also exposed to mercury vapors. Vaccinations expose children to mercury even though the blood levels of mercury do not rise above the safe recommended levels [6]. Mercury in high doses is a neurotoxin [7]. The experimental studies, mainly in animals, showed that mercury also affects the immune system by inducing lymphoproliferation and autoimmunity [2, 4]. The salient features of the mercury-induced autoimmunity are the ANA and antilaminin antibodies [2, 4]. Unlike animals, the mercury-induced autoimmune reaction is rare in humans; however, when it occurs it causes the nephrotic syndrome [2] accompanied by positive titers of antiglomerular basement membrane antibodies, specifically the antilaminin antibodies. Autistic children are not known to have the nephrotic syndrome and, as described here, they had normal titers of antilaminin antibodies, suggesting the absence of a mercury-induced autoimmune reaction in autistic children. Mercury exposure in industrial workers induces antibodies to neural antigens, mainly the neurofilament 68-kd protein [3]. In contrast, the antibodies to antineurofilament 68-kd protein are virtually nonexistent in autistic children, who primarily harbor antibodies to myelin basic protein [8] but sometimes also have antibodies to glial filament protein and neuron-axonal filament 200-kd protein [11]. Furthermore, it should be clearly pointed out that the mercury-exposed industrial workers very seldom harbor antibodies to myelin basic protein or neuron-axonal filament 200-kd protein [3]. Indeed, this is an important distinction between the mercury-exposed autoimmunity and the idiopathic autoimmunity in autism. The latter might be triggered by a virus infection, perhaps an atypical measles infection such as the one reflected by abnormal virus serology in children with the disorder [8–10].

Based on anecdotal reports of similarities between the clinical signs of mercury toxicity and the behavioral manifestations of autism, the disorder has been hypothesized

as an expression of mercury poisoning resulting from thimerosal in childhood vaccines [1]. However, a careful review of the literature resolved major differences between the neurological manifestations of autism and the clinical signs of mercury toxicity, which led to the conclusion that thimerosal and autism are not linked [5]. Additionally, as pointed out herein, there are some important differences between immunological abnormalities in autism and the immune aberrations of mercury exposure. To that end, we conducted the first experimental study and as described here the outcome of this study did not render support for a speculative relationship between mercury exposure and the autoimmune phenomenon in autism. Accordingly, we think that the chance of setting up an autoimmune response from exposure to small amounts of mercury is rather rare, unless some other factor concurrently contributes and increases the susceptibility to mercurial effects. Although our negative finding is quite compelling, we do not think it is sufficient to rule out such a relationship completely. Instead, we feel our study will encourage more experimental studies to establish whether or not thimerosal-derived mercury can cause autoimmune manifestations in children with autism. In view of the possibility that autism might be a multifactorial disorder, we believe that it is instructive to design and conduct experimental studies to investigate if this form of mercury can also trigger other causative mechanisms that might occur independently and/or concomitantly with the autoimmune mechanism. Naturally, more experimental research is needed on this very important topic of mercury and autism.

Acknowledgments

We thank the Duddley Dougherty and the BHARE Foundations and Autism Research Institute for their grant support. The authors did not have any conflict of interest in this regard.

References

1 Bernard S, Enayati A, Redwood L, Roger H, Binstock T. Autism: a novel form of mercury poisoning. Med Hypotheses 56:462–471;2001.
2 Bigazzi PE. Autoimmunity induced by metals. In: Lahita RG, Chiorazzi N, Reeves WH, eds. Textbook of the Autoimmune Diseases. Philadelphia, Lippincott Williams & Wilkins, 753–783; 2000.
3 El-Fawall HA, Watreman SJ, De Feo A, Shamy MY. Neuroimmunotoxicology: humoral assessment of neurotoxicity and autoimmune mechanisms. Environ Health Perspect 107 (suppl 5):767–775;1999.
4 Hultman P, Hansson-Georgiadis H. Methyl mercury-induced autoimmunity in mice. Toxicol Appl Pharmacol 154:203–211;1999.
5 Nelson KB, Bauman ML. Thimerosal and autism? Pediatrics 111:674–679;2003.
6 Pichichero ME, Cernichiari E, Lopreiato J, Treanor J. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. Lancet 360: 1737–1741;2001.
7 Schettler T. Toxic threats to neurologic development of children. Environ Health Perspect 109(suppl 6):813–816;2001.
8 Singh VK. Neuro-immunopathogenesis in autism. In: Berczi I, Gorczynski RM, eds. NeuroImmune Biology: New Foundation of Biology. New York, Elsevier, 443–454; 2001.
9 Singh VK, Jenson RL. Elevated levels of measles antibodies in children with autism. Pediatr Neurol 28:292–294;2003.
10 Singh VK, Lin SX, Newell E, Nelson C. Abnormal measles-mumps-rubella antibodies and CNS autoimmunity in children with autism. J Biomed Sci 9:359–364;2002.
11 Singh VK, Warren RP, Averett R, Ghazziuddin M. Circulating autoantibodies to neuronal and glial filament proteins in autism. Pediatr Neurol 16:88–90;1997.