



Elevated Levels of Measles Antibodies in Children with Autism

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Virus-induced autoimmunity may play a causal role in autism. To examine the etiological link of viruses in this brain disorder, we conducted a serological study of measles virus, mumps virus, and rubella virus. Viral antibodies were measured by enzyme-linked immunosorbent assay in the serum of autistic children, normal children, and siblings of autistic children. The level of measles antibody, but not mumps or rubella antibodies, was significantly higher in autistic children as compared to normal children ($p = .003$) or siblings of autistic children ($p \leq .0001$). Furthermore, immunoblotting of measles vaccine virus showed that the antibody was directed against a protein of approximately 74 kd molecular weight. The antibody to this antigen was found in 83% of autistic children but not in normal children or siblings of autistic children. Thus autistic children have a hyper-immune response to measles virus, which in the absence of a wild-type measles infection might be a sign of an abnormal immune reaction to the vaccine strain or virus reactivation. © 2003 by Elsevier Science Inc. All rights reserved.

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Introduction

Autism is a complex disorder of the central nervous system (CNS), manifesting both neurological as well as behavioral impairments. The disorder causes severe deficits of higher mental functions such as social interaction, language, communication, imagination, and cognition. The etiology and pathogenesis of the disorder is not well known or established. Current theories include genetic factors, immune factors, viral factors, neural factors, and

yet other unidentified factors. To that end, we focused on autoimmune mechanism of pathogenesis for autism [1-4]. Because viruses are common trigger agents for autoimmune diseases, we hypothesized that a virus-induced autoimmune response may play a causal role in autism [5,6]. Since viral studies are extremely scarce in autism, we conducted a serological study of three viruses, namely measles virus, mumps virus, and rubella virus. In this communication, we describe elevated levels of measles antibodies in autistic children, possibly as a consequence of a misguided immune response to measles vaccine.

Materials and Methods

We conducted a serological study of measles virus (MV), mumps virus (MuV), and rubella virus (RV) in autistic children and control children. The study included 88 autistic children (aged 3-10 years), 32 normal children (aged 4-10 years) and 15 siblings of autistic children (aged 4-11 years). However, because of our limited resources, not all sera were tested for all three viruses. The samples for analysis were randomly selected and tested in a blinded fashion to avoid inherent bias. As described previously [1-3], the clinical diagnosis of autism relied essentially on standard DSM-III-R criteria of the American Association of Psychiatrists (APA), Washington, DC. The Institutional Review Board (IRB) reviewed and approved our research protocol that involved the use of human serum samples. At the time of blood draw or a minimum of two weeks before the blood draw, none of the patients or controls was taking any prescription medications such as antipsychotic or neuroleptic drugs. Because of our ongoing research of autoimmunity in autism [3-5], we used previously collected sera that were kept frozen at -20°C . In this study, we included children with a firm diagnosis of autism only. According to individual records, all children in the study had their measles-mumps-rubella (MMR) immunization but none had any history of a wild-type infection to measles, mumps, or rubella virus. Viral antibodies were measured by using commercially available ELISA kits (Sigma Diagnostics, St. Louis, MO). These assays were performed essentially according to technical instructions of the manufacturer of the ELISA kits. Subsequently, the antigenic detection of measles virus was attempted by immunoblotting that was performed according to our published report [4-6]. The source of the virus was measles virus vaccine (MVV) (Merck & Co, Inc., West Point, PA); this choice was made because we did not have access to proper facilities for handling the wild strain of measles virus. Briefly, the viral proteins were separated in 12%

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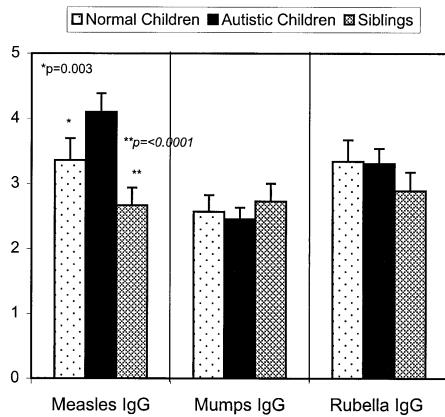


Figure 1. ELISA detection of viral antibodies in autism. Antibody levels are characterized for measles virus, mumps virus and rubella virus in autistic children (dotted bars), normal children (solid bars), and siblings of autistic children (shaded bars). The "n" values were: (a) 87 autistic children, 32 normal children and 14 siblings of autistic children for measles antibodies; (b) 30 autistic children, 32 normal children and 11 siblings of autistic children for mumps antibodies; and (c) 74 autistic children, 45 normal children and 15 siblings of autistic children for rubella antibodies. Statistically, as evaluated by the Student's t test, the measles antibody level was significantly increased in autistic children.

Ready Gels (Bio-Rad Labs, Richmond, CA) by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). They were transferred to nitrocellulose membranes (NCM) by double sandwich technique, followed by blocking with 1% bovine serum albumin in TBS buffer. The NCM were stored at room temperature. For immunoassay, 3-4 mm wide blots were cut and incubated with human sera for 1 hour. After 4 washings with TBST (TBS buffer containing 0.05% Tween-20), the blots were incubated for 1 hour with goat anti-human polyvalent-alkaline phosphatase conjugate. After 4 washings, the blots were developed in substrate solution by using AP substrate kit (Bio-Rad Labs, Richmond, CA). A reaction was scored positive whenever a purplish-blue band was seen. The statistical significance of data was evaluated by the Student's t test using Statview software for the Macintosh computer.

Results and Discussion

Serologically, the quantitative levels of viral antibodies are described in Figure 1. It should be noted that the measles antibody level was significantly ($p = .003$) higher in autistic children as compared to normal children. However, in these two groups of children, the level of mumps antibodies or rubella antibodies did not attain statistical significance; the p values were 0.759 and 0.879 for mumps antibodies and rubella antibodies, respectively. Moreover, a similar result was found when the comparison was made between autistic children and siblings of autistic children, i.e., autistic children harbored significantly ($p \leq 0.0001$) higher levels of measles antibodies but not mumps or rubella antibodies when compared to siblings of autistic children. Furthermore, the immunoblotting analysis of antigens immunopositive for measles antibodies is illustrated in Figure 2. The antibody in the autistic serum recognized a protein of approximately 74 kd molecular weight in the MVV blot (Figure 2, right panel 4 blots) but the normal serum did not show this antibody reaction (Figure 2, left panel 4 blots). While not revealed here, the sera of siblings of autistic children were also negative.

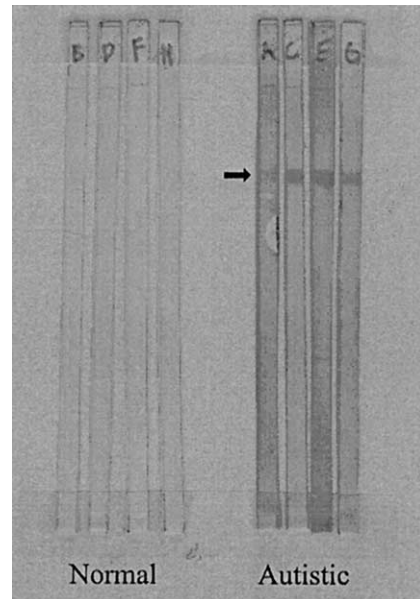


Figure 2. Immunoblotting of measles virus vaccine (MVV). Photograph showing representative immunoblots of measles antibody reaction in normal children and autistic children. As indicated by an arrow, the immunopositive reaction was detected with serum of autistic children (lanes A, C, E and G representing four children) but not the serum of normal children (lanes B, D, F, and H representing four children) with MVV protein of approximately 74 kd molecular weight.

After immunoblot screening of sera, we found that 43 of 52 (83%) autistic children, but none of the 30 normal children or 15 siblings of autistic children, had these antibodies to MVV. Since autistic children harbored these antibodies but control children did not we think they are abnormal or inappropriate antibodies to measles vaccine.

Autism is an idiopathic disorder of unknown etiology. We recently described a neuro-autoimmune hypothesis, which states that an autoimmune reaction to brain secondary to a viral infection may play a pathogenic role in autism [4]. Numerous studies support this idea. Autistic children have organ-specific autoantibodies, in particular autoantibodies to myelin basic protein (MBP) of the brain myelin [1,3-6]. As described elsewhere [1-8], autism is associated with immunogenetic susceptibility factors, family history of autoimmune diseases, abnormal immune regulation especially of T helper (CD4+) and natural killer (NK) cells, imbalance of Th-1/Th-2 cytokines that are known to induce autoimmune diseases, microbial factors such as viruses, and responsiveness to immune therapy. Collectively, therefore, these findings support the idea that autoimmunity plays a causal role in autism [1-6].

Autoimmune diseases are generally believed to be of viral origin. However, the viral studies in autism are scarce. Several years ago, autistic characteristics were described in some children with congenital rubella [9] and congenital CMV [10]. Recently, we found a serological association between measles virus and brain autoantibodies, and thereby we postulated an etiological link of this virus with autism [4-6]. Other researchers have recently detected the presence of measles virus in the peripheral

mononuclear cells of autistic children [11]. Serological data described here showed a significant increase of measles antibody in autistic children but the increase was not found for two other viruses (mumps and rubella) that we studied. In this regard, it is important to note that the serology for HHV-6 and CMV also did not differ between autistic children and normal children [4]. Thus autistic children have a hyper-immune response to measles virus specifically, but not to other viruses such as mumps, rubella, HHV-6 or CMV. Taken together, these findings suggest an etiological role of measles virus with the disorder.

Furthermore, in an attempt to determine antigenicity of the virus, we found that measles antibodies were immunopositive to measles virus vaccine (MVV), specifically to a protein of approximately 74 kd molecular weight. For this purpose, we did not use the wild strain of virus because we did not have proper laboratory facilities to handle the live strain of the virus. However, we are planning to address the vaccine versus the wild strain issue in near future. The nature of the MVV-derived protein is presently not known but its molecular weight appeared to resemble the molecular weight of hemagglutinin (HA) antigen of measles virus; the initial characterization of MMR vaccine-derived proteins was recently described elsewhere [6]. Thus the hyper-immune response to measles virus could possibly be directed towards the HA antigen; however, more research is needed to firmly establish this result. Moreover, it should be pointed out that none of the autistic children in our study had any history of a measles rash or wild type measles infection but they all have had their immunization with measles vaccine MMR [4-6]. This vaccine in a small population of genetically predisposed children may perhaps manifest an atypical measles infection that does not yield a clinical rash but produces neurological symptoms similar to those seen in children with autism. Alternatively, a mutant measles infection, similar to the one recently described [12], might exist in autistic children. While more research is necessary to uncover the etiology of autism, the hyper-

immune response to measles virus might indicate virus reactivation that triggers a misguided humoral immune response in children with the disorder.

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