

## **AUTISM, VACCINES, AND IMMUNE REACTIONS**

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### **1. Introduction**

Autism is the fastest-growing developmental disability in children today. Millions are affected worldwide, and the numbers are rising sharply to epidemic levels. Autism causes neurological and behavioral deficits in children, impairing their ability for social interaction, language, communication, cognition, and imagination. Autism is an idiopathic disorder of unknown etiology. Current theories include genetic factors, immune factors, viral factors, neural factors, and yet other unidentified factors. For last 10-12 years, I focused on autoimmune mechanism of pathogenesis and autoimmune therapy for patients affected with the disorder. I started out working on autism with the idea of "Autism as an Autoimmune Disorder" and my hope was to use autoimmunity as a target of drug development for treating autism [1-3]. Indeed, this is happening today [4-6]. In my presentation today, I will be speaking on the topic of "autism, vaccines, and immune reactions" and to examine the possibility of a causal link between vaccines and autism.

I view autism as a very complex disorder with different subsets and one such subset might be autoimmune in origin. I have studied autism as an autoimmune disorder, in which viral-autoimmune interplay may lead to pathological changes in the central nervous system (CNS). The essence of my "Autoimmune Hypothesis" is that a virus-induced autoimmune response to developing brain myelin may impair anatomical development of neural pathways in autistic children [3,4]. This line of thinking relies on the fact that the speed of nerve-impulse transmission depends essentially on structural properties of the insulating myelin sheath, connecting nerve fibers, and axon diameter. The anatomical changes could ultimately lead to life-long disturbances of higher mental functions such as learning, memory, communication, social interaction, etc. Fundamentally, therefore, I think that autism can be treated successfully using some of the therapies proven effective in treating other autoimmune diseases. To that end, however, the complete identification and characterization of autoimmune pathology in autism is of utmost importance today.

### **Autoimmune Hypothesis**

**Environmental Factors (viruses/vaccines) → Immune Dysfunction →  
Autoimmunity to Brain → Autism**

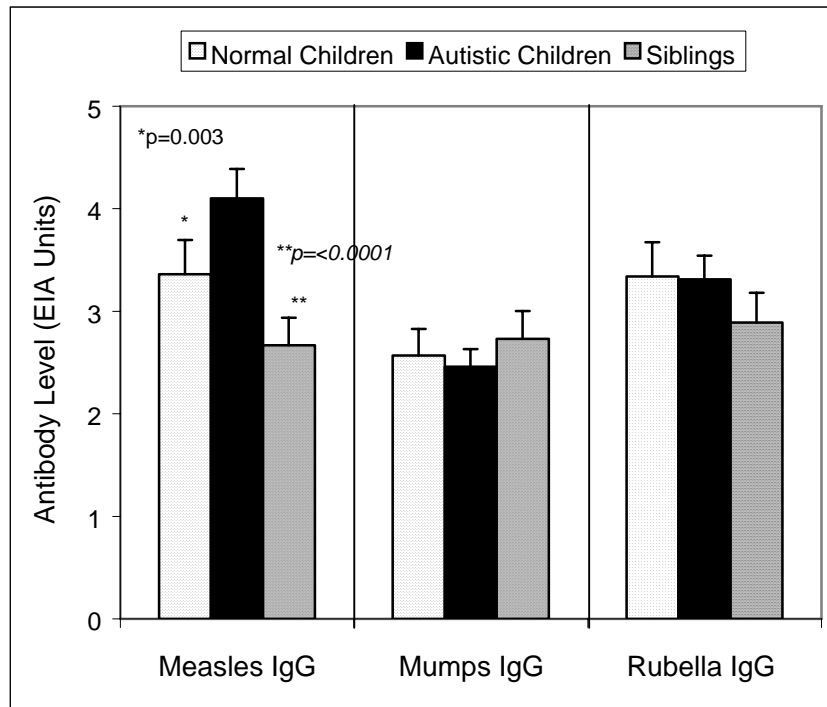
**2. Viruses as causal factors in autism: Measles is likely an etiological agent.**

Leading scientists in the field commonly believe that the viral infections trigger autoimmune responses and eventually lead to organ-specific autoimmune diseases. In autism, the trigger mechanism is not known but viral infections have been suspected. Viruses can enter the brain through the nasopharyngeal membranes or induce an autoimmune response against the brain, thereby impact the development of the central nervous system (CNS). Since the onset of the disorder is quite early on in life, viruses might serve as teratogens (agents that cause developmental malfunctions) etiologically linked to autism. Children with congenital rubella had certain autistic-like behaviors. Some autistic children did not make antibodies to rubella vaccine even after the repeated rubella immunization. Few cases of autism have also been described among children with congenital cytomegalovirus (CMV) infection.

Recently, we took a novel approach of studying viral etiology in autism [7-9]. We raised two simple questions: First, do autistic children harbor abnormal virus serology (antibody levels) and, secondly, is there a correlation between virus serology and brain antibodies. We studied immune response to viruses by measuring the level of their antibodies. For this purpose, we measured antibodies to five viruses: Measles virus, mumps virus, rubella virus, CMV, and human herpesvirus-6 (HHV-6). To our surprise, we found that the antibody level of only the measles virus, but not of the other viruses tested, was significantly higher in autistic children than the normal children [reference #4 and #9; Table 1 and Fig. 1]. In addition, we found an interesting correlation between measles antibody and brain autoimmunity, which was marked by myelin basic protein (MBP) antibodies. These two immune markers correlated in 90% or greater autistic children, suggesting a causal link of measles virus with autoimmunity in autism. But the serology to other viruses and other brain autoantibodies did not show this correlation. This was a very important finding that prompted us to postulate a temporal link of measles virus in the etiology of the disorder [Singh et al., 1998; reference #7]. To that end, it is also noteworthy that the immune manifestations of measles virus infection are quite similar to the immune abnormalities in autistic children, indirectly pointing to an etiological link of measles infection in autism.

Table 1. Viral antibodies in the sera of autistic children

Virus antibody	Subject group	EIA Units (mean ± SD)	p value
CMV-IgG	Autistic (n=30)	0.23 ± 0.32	0.37
	Normal (n=30)	0.28 ± 0.46	
HHV-6-IgG	Autistic (n=45)	2.18 ± 5.35	0.459
	Normal (n=37)	1.52±0.64	
Rubella-IgG	Autistic (n=31)	3.59 ± 1.19	0.076
	Normal (n=12)	2.90 ± 0.81	
Mumps-IgG	Autistic (n=30)	2.57 ± 1.50	0.759
	Normal (n=32)	2.46 ± 1.31	
<b>Measles-IgG</b>	<b>Autistic (n=42)</b>	<b>3.83 ± 1.23</b>	<b>0.004</b> <b>(Significant)</b>
	<b>Normal (n=26)</b>	<b>3.08 ± 0.45</b>	



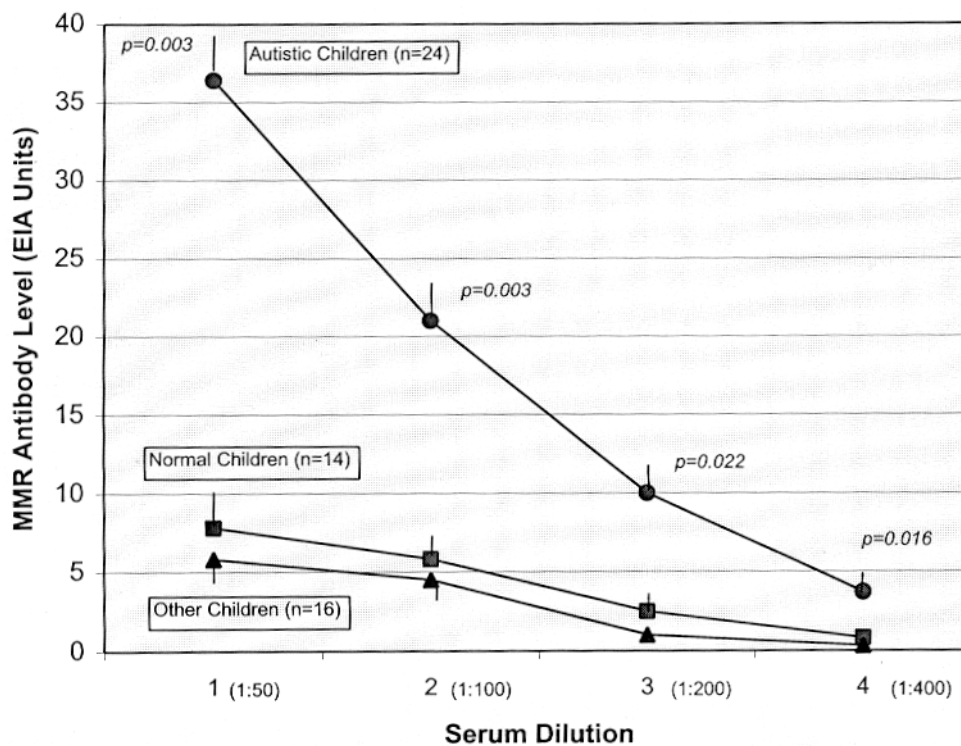
**Figure 1.** Viral antibody levels are shown for measles virus, mumps virus and rubella virus in autistic children (n=87, dotted bars), normal children (n=32, solid bars) and siblings of autistic children (n=14, shaded bars). **Note: As evaluated by the Student's t test, the measles antibody level was significantly ( $p \leq 0.05$ ) increased in autistic children.**

### **3. Vaccines as risk factors in autism: MMR could potentially cause autism via an autoimmune mechanism.**

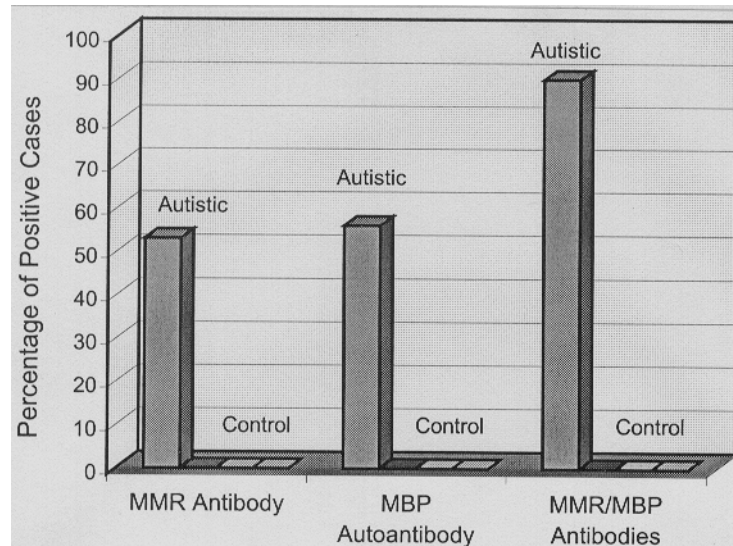
Although there is very little experimental data, parents of autistic children commonly report the onset of autistic characteristics shortly after immunization with measles-mumps-rubella (MMR) and/or diphtheria-pertussis-tetanus (DPT) vaccines. This subset is sometimes referred to as "Autistic Regression." This population includes approximately 85% of autistic children quite commonly with a new diagnosis of the disorder. The remainder 15% of children either does not have any history of adverse vaccine reaction or they have diagnosis of autism without the vaccines. This information, albeit anecdotal, was quite important when we expanded our investigation further by using the MMR vaccine (see below).

To examine vaccines as risk factors in autism, we conducted a study of serology (antibody levels) to three vaccines: MMR, DPT and DT (diphtheria-tetanus). Once again, we raised the same two questions: First, do autistic children harbor abnormal vaccine serology (antibody levels) and, secondly, is there a correlation between vaccine serology and brain autoantibodies. Through our experimental research [8], we found that the level of MMR

antibodies was significantly higher in autistic children as compared to normal children or other disease children [Fig. 2]. Moreover, it is noteworthy that autistic children exhibited a very high degree of specificity for MMR antibodies, similar to our previous finding for measles antibodies, which is of paramount importance in establishing an etiological role of MMR in autism. Furthermore, we characterized that this abnormal MMR serology was due to antibodies to measles subunit but not the mumps or rubella subunit of the trivalent vaccine MMR [8]. The same result was also found when we used monovalent measles vaccine in lieu of the trivalent MMR vaccine, furthermore pointing to a problem of only the measles subunit [9]. Once again, there was a positive correlation (90% or greater) between MMR antibody and MBP autoantibody [Fig. 3; reference #8]. These findings led me to speculate that the measles subunit of the MMR vaccine might trigger an autoimmune reaction in a significant number of autistic children [7-9]. While more research is necessary, I think this is an excellent working hypothesis to explain autoimmune subset of autism and it may also help us understand why some children show “autistic regression” after MMR immunization. To that end, it is also important to note that the MMR vaccine induces a Th-1 cellular response [10], an immune response that is also found in autistic children [6,11].



**Figure 2.** MMR antibodies in autism. At four serum dilutions, the MMR antibody levels are shown for autistic children (n=24, solid circles), normal children (n=14, solid squares), and other disease children (n=16, solid triangles). **Note: MMR antibody level was significantly ( $p \leq 0.05$ ) increased in autistic children.**



**Figure 3.** MMR and MBP antibodies in autistic children. **Note that 90% or more correlation was found between MMR and MBP antibodies in autistic children** (vertical bars) but not in controls that included normal children (baseline box 1), normal siblings (baseline box 2), and other disease children (baseline box 3).

#### **4. Immune reactions in autism: Autoimmunity is the core of the problem.**

Several groups have demonstrated abnormal immune reactions, in particular autoimmunity, in children with autism and related spectrum disorders. Autoimmunity is an abnormal immune reaction in which the immune system goes haywire and reacts abnormally against body's own organs, and the end result is an autoimmune disease. Several factors contribute to autoimmune diseases. Microbes such as viruses can trigger autoimmune diseases. They are generally linked to certain genes that control immune responses. They cause immune abnormalities of white blood cells (WBC), in particular that of T cells, B cells and NK cells. They induce the production of pathogenic antibodies, especially the organ-specific autoantibodies. They involve hormonal factors. And they generally show a gender preference. This is also the case with autism, which means that several autoimmune factors have also been found in autistic children [for various citations see references 1-9]. Some of the important autoimmune factors in autism are:

1. Autism is commonly associated with microbial infections, in particular viral infections.
2. Autistic patients have immune abnormalities, especially those that characterize an autoimmune reaction in a disease.
3. Autism shows inappropriate immune responses to vaccines, in particular MMR.
4. Autism displays increased frequency for immune response genes (e.g., HLA, C4B null allele or extended haplotypes) that render susceptibility to autoimmune diseases.
5. Autism involves a gender factor as it affects males about four times more than females.
6. Autism has a family history of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and diabetes.
7. Autism also involves a hormonal factor, for example secretin and endorphins.
8. Autistic patients respond well to immune modulation therapy (IMT).

### **5. Mercury and autism: *Mercury is not related to autoimmunity.***

Because both autism and mercury exposure involve autoimmunity, mercury has recently been proposed as an environmental risk factor in autism. However, the laboratory analysis showed that the blood levels of mercury do not rise above safe levels in infants and children receiving thimerosal-containing vaccines [12]. We hypothesized that if autism involved a connection between mercury exposure and autoimmunity then autistic children should harbor elevated levels of mercury-induced autoimmune markers, namely the antinucleolar antibodies and antilaminin antibodies. So, we recently conducted a pilot study of these two autoimmune markers in autistic children and normal children. The results of our experimental study, for the first time, showed that the distribution of these two markers did not change in autistic children. Quite plainly, mercury is not a risk factor for autoimmunity in autism [Our research in progress].

### **6. Immune Modulation Therapy (IMT) in Autism**

Accumulating evidence suggest that autoimmunity plays a key role in the pathogenesis of autism. The idea that autism is an autoimmune disorder is further strengthened by the fact that autistic patients respond well to treatment with immune modulating drugs [4-6]. Immune interventions can produce immune modulation – a state of suppression or stimulation. Since autistic patients do not show a classical primary immunodeficiency, simply boosting their immunity is not a good strategy. However, they do have immune abnormalities and therefore, depending on the nature of the immune abnormality, the goal of IMT should be to normalize or reconstitute the immune function. This will permit a more balanced immune response, avoiding major fluctuations of overt immune activity that could be detrimental to the patient. The IMT should always be given in consultation with a physician, preferably a clinical immunologist, allergist or hematologist. For autism, the recommended list of IMT includes steroid therapy, immunoglobulin therapy, oral autoantigen therapy, transfer factor therapy, immunomodulating drugs, and plasmapheresis.

### **7. Summary and concluding remarks**

1. Considering an estimated population of 500,000 Americans with autism (not including all spectrum disorders), I think that between 250,000 to 350,000 autistic patients could benefit directly from autoimmunity research today.
2. The current genetic research estimates that no more than 10% of all autistic cases are genetic in origin. Simply put, the remainder 90% of autistic cases is sporadic with a non-genetic etiology. I tend to think that the sporadic form is by and large an “acquired” subset involving autoimmunity. This subset is likely triggered by a virus, possibly measles virus or MMR vaccine. I recently designated this subset as an “Autoimmune Autistic Disorder (AAD)” – a term coined to describe autoimmune subset of autism (Singh VK: May 1, 2003). I think that the autoimmunity research has a global impact for treating autism worldwide hence the physicians and researchers should pay a closer attention to autoimmunity research in autism.

3. Based upon our experimental research, it is plausible to postulate that an atypical measles infection that does not produce a typical measles rash but manifests neurological symptoms might be etiologically linked to autoimmunity in autism. The source of measles virus could potentially be MMR vaccine or a mutant measles strain, but more research is necessary to establish either of these two possibilities.
4. Fundamentally, I tend to think that autistic children have a problem of their immune system, which is the “faulty immune regulation.” Hence they have abnormal immune reactions to measles virus and/or MMR vaccine.
5. I am a strong advocate of immunization program worldwide, chiefly because the vaccines are the best preventive measures against deadly infections available to mankind today. So, I am not an anti-vaccine person. I do believe however that the safety of vaccines must be as absolute as humanly possible because they are administered into healthy children, adults and elderly.
6. **To conclude**, since everything changes with time, I firmly believe that it is time to re-evaluate the safety of vaccines and the manner in which we practice immunizations. Vaccines are well known to cause numerous adverse reactions in humans and no matter how rare they might be it is time to pay a closer attention to them. I don't think the epidemiological studies will suffice the purpose but the laboratory-based experimental research is urgently needed. We need new policies simply because the existing policies are not in line with our modern knowledge of human immunology, virology, and genomics. This is clearly exemplified by our experimental approach involving laboratory techniques that did not exist 30-40 years ago when the vaccines were originally developed. Indeed, there is persuasive evidence to suggest that the MMR vaccine could potentially cause autism or a regressive form thereof in a significant number of children. At this juncture, I would also like to recommend a new policy of **“Testing immunity before vaccination or immunization”** that should help identify immunocompromised children who otherwise might react adversely to vaccines. The cost should not deter us from implementing this policy especially when the lives of hundreds and thousands of children and their families are concerned.

## **8. Financial support disclosure**

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## 9. Relevant references

1. **Singh VK.** Brain, Autoimmunity and autism. *Invited presentation at the Annual Conference of the Long Beach Chapter of Autism Society of America (ASA)*, Long Beach, California; October 13-14, 1995.
2. **Singh VK,** et al. Immunodiagnosis and immunotherapy in autistic children. *Presented at the International Congress of Neuroimmunology*, Philadelphia, Pennsylvania; Sept. 8-11, 1987.
3. **Singh VK,** et al. Antibodies to myelin basic protein in children with autistic disorder. *Brain Behavior and Immunity* 7: 97-103 (1997).
4. **Singh VK.** Neuro-immunopathogenesis in autism. **In:** *NeuroImmune Biology: New Foundation of Biology*, Vol. 1: 443-454 (2001).
5. **Singh VK.** Immunotherapy for brain disease and mental illnesses. *Progress in Drug Research* 48: 129-146 (1997).
6. **Singh VK.** Cytokine regulation in autism. **In:** *Cytokines and Mental Health*, Edited by Z. Kronfol (2003) pp. 369-383, Kluwer Acad. Publishers, Boston, MA.
7. **Singh VK,** et al. Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism. *Clin. Immunol. Immunopathol.* 89: 105-108 (1998).
8. **Singh VK,** et al. Abnormal measles-mumps-rubella antibodies and autoimmunity in children with autism. *Journal of Biomedical Science* 9: 359-364 (2002).
9. **Singh VK** and Jensen RL. Elevated levels of measles antibodies in children with autism. *Pediatric Neurology* 28: 292-294 (2003).
10. **Pabst HF,** et al. Kinetics of immunologic responses after primary MMR vaccination. *Vaccine* 15: 10-14 (1997).
11. **Singh VK.** Plasma increase of interleukin-12 and interferon-gamma: Pathological significance in autism. *Journal of Neuroimmunology* 66: 143-145 (1996).
12. **Pichichero ME,** et al. Mercury concentrations and metabolism in infants receiving vaccines containing thiomersal: a descriptive study. *Lancet* 360: 1737-1741 (2001).