

Phenotypic expression of autoimmune autistic disorder (AAD): A major subset of autism

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BACKGROUND: Autism causes incapacitating neurologic problems in children that last a lifetime. The author of this article previously hypothesized that autism may be caused by autoimmunity to the brain, possibly triggered by a viral infection. This article is a summary of laboratory findings to date plus new data in support of an autoimmune pathogenesis for autism.

METHODS: Autoimmune markers were analyzed in the sera of autistic and normal children, but the cerebrospinal fluid (CSF) of some autistic children was also analyzed. Laboratory procedures included enzyme-linked immunosorbent assay and protein immunoblotting assay.

RESULTS: Autoimmunity was demonstrated by the presence of brain autoantibodies, abnormal viral serology, brain and viral antibodies in CSF, a positive correlation between brain autoantibodies and viral serology, elevated levels of proinflammatory cytokines and acute-phase reactants, and a positive response to immunotherapy. Many autistic children harbored brain myelin basic protein autoantibodies and elevated levels of antibodies to measles virus and measles-mumps-rubella (MMR) vaccine. Measles might be etiologically linked to autism because measles and MMR antibodies (a viral marker) correlated positively to brain autoantibodies (an autoimmune marker)—salient features that characterize autoimmune pathology in autism. Autistic children also showed elevated levels of acute-phase reactants—a marker of systemic inflammation.

CONCLUSIONS: The scientific evidence is quite credible for our autoimmune hypothesis, leading to the identification of autoimmune autistic disorder (AAD) as a major subset of autism. AAD can be identified by immune tests to determine immune problems before administering immunotherapy. The author has advanced a speculative neuroautoimmune (NAI) model for autism, in which virus-induced autoimmunity is a key

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player. The latter should be targeted by immunotherapy to help children with autism.

KEYWORDS: autism, viruses, autoimmunity, CNS infections, immunotherapy, developmental disorders, metal allergy, neurotoxicity, immunotoxicity

INTRODUCTION

Although autism was first described by American psychiatrist Leo Kanner more than 60 years ago, the cause and treatment of this brain disorder still remains poorly understood. Now, autism is no longer regarded as a simple developmental disorder but rather a biological disorder of complex etiology and heterogeneity.¹⁻⁴ Autism is defined not by etiology or pathology but by the presence of a constellation of behavioral characteristics that accompany a particular developmental course, with evidence of developmental delay within the first 3 years of life. Typically, autism is characterized by “qualitative deficits” in 4 major categories: (1) deficits of developmental rates and/or profiles, (2) deficits of responses to sensory stimuli, (3) deficits of speech, language, and communication capabilities, and (4) deficits of social interactions and/or manners of relating to other people. Although the diagnosis of autism is made during early childhood, the disorder continues to persist well into adulthood, eventually becoming a lifelong neurodisability. Until about 10 years ago, the prevalence of autism in America was 4 to 5 children per 10,000 births, but the number of autism cases has increased dramatically.^{5,6} The U.S. Centers for Disease Control and Prevention recently reported that as many as 1 in 125 to 150 children are diagnosed with autism—a rate that has increased at least 10 times in the last 10 years. Autism affects boys about 4 times more often than girls, implying that the prevalence would be much higher in males, possibly reaching a rate of 1 in 84 boys. Furthermore, although the precise data are scarce, a similar trend of a sharp rise in rates of autism spectrum disorders (ASD) has been described in other countries, for example, an estimated 200,000 children in Canada, 1 to 2 million children in India, and 1.5 to 3 million children in China.

An estimated 10 or more genes have been implicated in ASD,⁷ but their association is only indirect, and no single gene has been specifically identified for autism. Based on this and other epidemiologic considerations, we had suggested that genetic factors would

account for only a smaller percentage ($\leq 10\%$) of autism cases, whereas the remaining, larger percentage ($\geq 90\%$) of cases would be sporadic due to nongenetic factors.⁴ The sporadic form might be acquired from exposure to environmental factors such as viruses, vaccines, or chemical toxins and other unknown factors. In this article, the author summarizes his laboratory research to date and reviews scientific data that lend credibility to a virus-induced autoimmune mechanism of pathogenesis for autism. The knowledge resulting from autoimmunity research has direct clinical relevance to the overall health and recovery of autistic children. Accordingly, the author suggests that autoimmunity is a very important target of therapeutic development for autism, and offers a novel approach to immunotherapy with use of natural immunomodulators.

Presentation of a new theory: Autoimmune mechanism of pathogenesis in autism

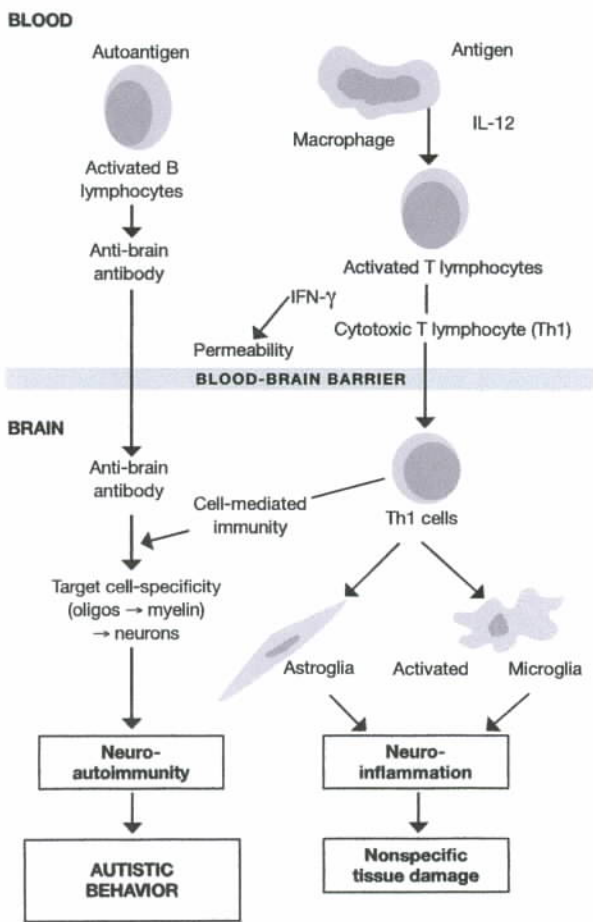
Autism is a very complex disorder that may possibly result from abnormal function of the neuroautoimmune (NAI) circuitry.¹ More than 20 years ago, we hypothesized that environmental factors such as a viral infection might cause autoimmunity to brain and thereby viral-immune interactions may lead to pathologic changes in the brain of children with autism.⁸⁻¹⁰ As outlined below, brain-specific autoimmunity could be responsible for neuropathology in autism.

Environmental Factors (virus) → Faulty Immune Regulation → Autoimmunity to Brain → Neuropathology in Autism

Several years ago, we hypothesized that a virus-induced autoimmune reaction to the developing brain, in particular the developing myelin sheath, may cause anatomic abnormalities of neural connections in the brains of children with autism.¹⁰ This is a very important event in the developing brain because the speed of nerve-impulse transmission depends essentially on structural properties of the insulating myelin sheath, connecting nerve fibers, and axon diameter. We postulated that a virus-induced immune assault might cause “nicks” or subtle changes in the myelin sheath.^{2,4,10} An autoimmune reaction to the developing myelin sheath could ultimately lead to lifelong impairments of higher brain functions, such as speech, language, communication, and social interaction as well as other neurologic symptoms that are commonly exhibited by children with autism.

FIGURE 1

Neuroautoimmunity (NAI) model of autism



Source: Reference 38.

Through extensive laboratory research, we have identified certain abnormalities of viral, immune, auto-immune, and neural factors in children with autism. It should also be noted that autism tends to show a family history of autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, and type 2 diabetes mellitus.^{11,12} Moreover, to explain the role of autoimmunity, we first described a speculative neuroautoimmunity (NAI) model of autism¹³ at a conference sponsored by the Autism Society of America.

According to this NAI model, a viral infection (foreign antigen) could trigger an autoimmune response by activating antigen-presenting cells (macrophages or dendritic cells) that, via interleukin-12 (IL-12) induction, would activate T lymphocytes. As depicted in

FIGURE 1, the viral infection appears to be a measles infection, possibly resulting from exposure to the measles-mumps-rubella (MMR) vaccine, but it could also be a latent or mutant measles strain.

T lymphocytes would be activated via production of interferon- γ (IFN- γ) and would change the cell permeability at the blood-brain barrier. This is because IFN- γ is the only known, naturally occurring molecule that induces the expression of Class I MHC antigens on the blood-brain barrier to cause permeability changes.¹⁴

After crossing the blood-brain barrier, the Th1 cells could recognize antibrain antibodies (anti-myelin basic protein [MBP]) that would be produced by autoantigen MBP-primed B lymphocytes and then circulate in the brain. Then, owing to their specificity for myelin sheath, the anti-MBP antibodies by themselves, or by interaction with antigen-specific T lymphocytes, could cause cell damage to oligodendrocytes, the myelin-synthesizing cells in the CNS. Consequently, the function of the oligodendrocytes would be altered to produce abnormal myelin sheath during brain development.

The entire cascade of events leading to a neuroautoimmune response would be responsible for abnormal neurodevelopment—in particular, the functionality of neural circuits or neural pathways would most likely be disrupted. Since the myelinated neuron-axon fibers have a specific regional distribution in the brain, the overall outcome would result in neurologic and behavioral manifestations that are characteristic of autism/ASD.

Alternatively, in the absence of highly specific brain autoantibodies (anti-MBP), the Th1 cells could interact with astrocytes and/or microglia to produce neuroinflammation, which would lead to only nonspecific tissue damage. Thus, the phenotypic expression of autistic behaviors would be the result of subtle anatomic changes in the brain myelin sheath.

One of the salient features of this model is the requirement for a high degree of specificity of antibrain antibodies (anti-MBP), candidate autoantigen (MBP), and targeted oligodendrocytes.²⁻⁴ Normally, myelin speeds up electrical impulses 20 to 100 times faster than unmyelinated axons. Furthermore, the oligodendrocyte precursor cells (OPCs) have recently been found to elicit electrical impulses.^{15,16} This finding suggests that myelin also plays a more direct role in electrical impulse transmission—a function very similar to nerve impulse transmission that, until now, was attributed to neurons only. In view of this novel finding, we hypothesize that the autoimmune reaction to myelin-

derived MBP could potentially impact and impair the structure and/or function of OPCs, oligodendrocytes, or developing myelin in the brains of autistic children.

Subject population and laboratory methods in our studies

All normal and autistic subjects in laboratory studies that we performed were at baseline, without any treatment with prescription medications, natural products, or nutritional supplements. This is a very important criterion for subject selection for studying immune system function, and attention should be paid when recruiting subjects for this purpose because prescription medications and/or natural supplements are well known to have immunomodulating properties that could alter the immune profiles of autistic children.

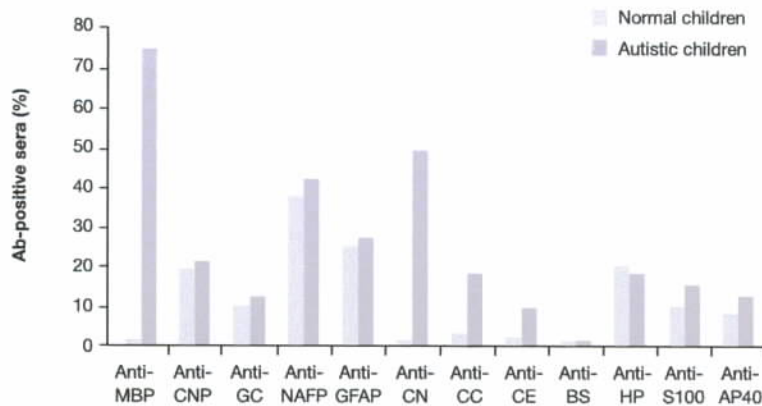
For our laboratory research, we enrolled autistic children and normal children and, in some studies, siblings of autistic children, children with other diseases, and, rarely, adults. In our study, we included only autistic children with a firm diagnosis of autism, but we excluded other diagnoses such as pervasive developmental disability (PDD), pervasive developmental disability not otherwise specified (PDD-NOS), and Asperger's disorder. All subjects were included and no one was denied participation in the study because of race, age, or gender factors. The clinical diagnosis of autism was made essentially according to DSM-IV criteria. Normal children were those having a history of physical health, without any sign of brain disease, mental illness, or any other known medical condition. We submitted our research protocol to the Institutional Review Boards of the University of Michigan and Utah State University and obtained their approval prior to blood collection. Whenever necessary, serum samples were stored frozen at -20°C , while keeping the freezing-thawing cycle to a minimum. Further details of our laboratory procedures and assay methods have been described in our other publications.^{2-4,9,10,17-24}

Immune findings in autism

Several lines of study have already yielded scientific

FIGURE 2

Distribution of serum autoantibodies to brain antigens in normal and autistic children



Ab: antibody; Anti-AP40: beta-amyloid protein (1-40) antibodies; Anti-BS: brain stem antibodies; Anti-CC: cerebral cortex antibodies; Anti-CE: cerebellum antibodies; Anti-CN: caudate nucleus antibodies; Anti-CNP: 2', 3'-cyclic nucleotide phosphohydrolase antibodies; Anti-GC: galactocerebroside antibodies; Anti-GFAP: glial filament acidic protein antibodies; Anti-HP: hippocampus antibodies; Anti-MBP: myelin basic protein autoantibodies; Anti-NAFP: neuron-axon filament protein antibodies; Anti-S100: S100 protein antibodies.

data that are beginning to fill in various steps of the NAI model, but more research is needed to identify and characterize this hypothetical model. Deriving from this model, however, it is believed that autism can be treated successfully with immunotherapies that have proven effective in treating other autoimmune diseases. In the case of autism, the organ-specific autoantibodies would be brain-specific autoantibodies. Indeed, a significant number of autistic children harbor autoantibodies to brain antigens (FIGURE 2). Of all the brain autoantigens tested, the most prevalent autoantigen is the CNS myelin-derived MBP,^{2,10} and the next suitable autoantigen was likely derived from the caudate nucleus.¹⁷ Pathologically, a very high prevalence rate (70% to 90% positive) of anti-MBP among autistic children would clearly suggest that MBP is a candidate autoantigen in autism.^{2-4,17-19}

Autoimmunity is an abnormal immune reaction in which the immune system becomes primed to react against body organs, and the net result is an autoimmune disease. The clinical presentation of autoimmune diseases involves several factors: environmental factors; genetic links, especially of immune response (IR) genes; immune abnormalities of thymus-derived immunoregulatory T cells; autoantibodies, especially organ-specific autoantibodies; gender factor for greater prevalence in

TABLE 1

Immune abnormalities in autistic children

1. In autism, microbial associations of certain viruses such as measles, rubella, and cytomegalovirus (CMV) have been shown.^{21,29,30}
2. Autistic children show a hyperimmune antibody response that recognizes hemagglutinin antigen (HA) of the measles virus, measles vaccine, and measles-mumps-rubella (MMR) vaccine, which abnormally correlates with autoantibodies to myelin basic protein (MBP).²¹⁻²⁴
3. Autism displays increased frequency of immune response (IR) genes, for example HLA antigens, C4B null allele, haplotype B44-SC30-DR4, HLA-C, and HLA-B1.³¹
4. Autistic patients have impaired humoral immunity, as shown by IgA deficiency; increased IgG3, and circulating antinuclear antibodies and immune complexes.^{4,32}
5. Autistic children have a deficiency of cellular immunity, as demonstrated by decreased lymphocyte counts, low T helper cell (CD4+) counts, reduced natural killer (NK) cell counts, suppressed mitogen-induced lymphocyte proliferation, and reduced function of NK cells.³²⁻³⁷
6. Autism involves a gender factor, affecting males about 4 times more often than females.
7. Autism often accompanies a family history of autoimmune diseases, for example, multiple sclerosis, rheumatoid arthritis, and type 2 diabetes mellitus.¹¹
8. Autism involves hormonal factors, eg, secretin, beta-endorphin, etc.²⁻⁴
9. Autistic patients have brain-specific autoantibodies; these are specific to brain MBP, which is a candidate autoantigen in autism.^{4,10,17}
10. Autistic children show an autoimmune reaction to specific proteins of the caudate nucleus, implying a pathogenic role for this particular brain region in autism.¹⁷
11. Autoantibodies to other brain antigens, like neuron-axon filament proteins (NAFP), serotonin receptor proteins, and galactocerebrosides, are also found but they are not specific to autism because they are also found in many normal children.^{2,18,19}
12. Autistic patients show immune activation as reflected by T-cell activation and elevation of autoimmunity-specific cytokines.^{3,9,38}
13. Autistic patients respond well to autoimmune therapy with oral autoantigen, transfer factor, and intravenous immunoglobulin.^{2-4,8,32,39-41}
14. Autistic children have acute-phase systemic inflammation, as demonstrated by elevated levels of serum C-reactive protein (CRP) and S100 proteins, which most likely precedes inflammation of the brain.²⁰
15. Autistic children have normal levels of metallothionein (MT) protein, and antibodies to MT (anti-MT) are also in the normal range.^{42,43}

males or females; hormonal factors; and therapeutic response to immunomodulating agents.²⁻⁴ Often, an acute-phase inflammatory response is associated with autoimmune diseases.

There is evidence for both systemic inflammation²⁰ and brain inflammation in autism.^{25,26} As summarized elsewhere,² anatomic and morphologic changes have been

found in the white matter of brains of children with autism. Afterward, the white matter changes were also found by magnetic resonance imaging (MRI).^{27,28} In this regard, we postulated that autoimmunity to brain myelin could possibly induce developmental changes of white matter, which is composed of predominantly myelinated nerve fibers. Although this is a good possibility, other biochemical mechanisms should also be explored.

Immune studies in laboratories around the world have shown the existence of autoimmune problems (TABLE 1) in children with autism/ASD.^{2-4,8,10,17-24,29-43} However, careful attention must be paid to subjects recruited for the study. As stated previously, to conduct immune studies, children in the study must be at baseline prior to the administration of any prescription medication and/or alternative treatment because these regimens are known to modify the function of the immune system.

What triggers autoimmunity in autism is not known, but there is scientific evidence to suggest that measles virus might be a culprit; however, other infectious agents should also be examined. Although autoimmunity is commonly triggered by viral infections, other environmental factors, such as heavy metals (eg, mercury), can also induce an autoimmune response in animal models. However, there is no human study that supports the idea of autoimmunity in autism from exposure to heavy metals like mercury.^{42,43} Based on these considerations, we explored

2 possibilities in autism: (1) virus-induced autoimmune reaction, and (2) heavy metal (mercury)-induced autoimmune reaction. They are described in the following section.

Virus serology (antibodies) in autism. To search for viruses as etiologic agents in autoimmune diseases,

TABLE 2

Serum level of antibodies to viruses in normal and autistic children

Virus antibody (units)	Measles	Mumps	Rubella	HHV-6	CMV	EBV		
						EA	EBNA	VCA
Normal children	3.3 ± 0.1 (n = 32)	2.5 ± 0.2 (n = 30)	3.2 ± 0.2 (n = 45)	1.6 ± 0.6 (n = 37)	0.28 ± 0.4 (n = 30)	0.5 ± 0.04 (n = 44)	1.2 ± 0.2 (n = 44)	1.8 ± 0.3 (n = 44)
Autistic children	4.2 ± 0.1^a (n = 87)	2.6 ± 0.3 (n = 32)	3.3 ± 0.1 (n = 74)	2.2 ± 5.3 (n = 45)	0.23 ± 0.3 (n = 30)	0.6 ± 0.04 (n = 44)	0.9 ± 0.2 (n = 44)	1.4 ± 0.2 (n = 44)
P value	.003^a	.76	.98	.5	.37	.76	.21	.15

CMV: cytomegalovirus; EA: early antigen; EBNA: Epstein-Barr nuclear antigen; EBV: Epstein-Barr virus; HHV-6: human herpesvirus-6; VCA: viral capsid antigen.

^aStudent t test was used to evaluate significance at a P value < .05.

2 types of experimental approaches have been used: (1) virus isolation (viral antigens), and (2) virus serology (viral antibodies). Initial attempts were made to isolate measles virus from peripheral blood mononuclear cells and gut biopsies^{44,45}; however, the results are quite controversial and nonconclusive.⁴⁶ In our own laboratory, we took the second approach and evaluated virus serology.

VIRUS SEROLOGY. Virus serology is commonly regarded as a highly reliable index of antibody response to viruses in humans. Thus, we measured serum levels of viral antibodies against 6 randomly selected viruses: measles virus (MV), mumps virus (MuV), rubella virus (RV), cytomegalovirus (CMV), human herpesvirus-6 (HHV-6), and Epstein-Barr virus (EBV). The laboratory data in TABLE 2 revealed the existence of a hyperimmune response to measles virus in children with autism. A vast majority of autistic children harbored significantly higher than normal levels of antibodies to measles virus, but the level of antibodies to the other 5 viruses did not significantly differ between autistic and normal children (TABLE 2). Because this was a highly select finding for measles virus specifically, we postulated that there might be a temporal association between measles virus and autism.²¹⁻²⁴ Furthermore, we found that autistic children showed a serologic association between measles virus and MBP autoantibodies, ie, the higher the measles antibody level, the greater the chance of MBP autoantibody (≥90% positive correlation). This association was not found for other viruses and other brain autoantibodies that were tested in our laboratory. Clearly, this was the first evidence ever for an etiologic association of measles virus to autoimmunity in autism.^{2,21-24}

Vaccine serology (antibodies) in autism. The review of the medical histories of children with autism/ASD who participated in the vaccine serology study did

TABLE 3

Serum level of antibodies to vaccines in autistic and normal children

Vaccine antibody (units)	MMR	DPT	DT	Hep B
Normal children	5.2 ± 0.3 (n = 40)	10 ± 0.6 (n = 34)	11 ± 0.8 (n = 34)	2.4 ± 0.3 (n = 53)
Autistic children	9.5 ± 0.1^a (n = 42)	11 ± 0.5 (n = 54)	12 ± 0.6 (n = 54)	2.0 ± 0.7 (n = 54)
P value	.001^a	.63	.81	.21

DPT: diphtheria-pertussis-tetanus; DT: diphtheria-tetanus; Hep B: hepatitis B; MMR: measles-mumps-rubella.

^aStudent t test was used to evaluate significance at a P value < .05.

not reveal any sign of a typical rubella rash. This means that a wild-type measles infection is rather unlikely to occur in these children. However, we considered a remote possibility of an atypical or asymptomatic measles infection, in the absence of a typical measles rash. Such an infection could either occur by a variant measles infection or it could be acquired from immunization with MMR vaccine. An atypical measles infection in the absence of a rash or unusual neurologic symptoms has recently been described to suggest the existence of a variant measles virus in humans.⁴⁷

Because we did not have laboratory facilities to handle wild measles strain, we decided to examine the possibility of an acquired measles infection from MMR vaccination. Thus, we performed serologic studies of antibodies to vaccines. We selected 4 vaccines: measles-mumps-rubella (MMR), diphtheria-tetanus-pertussis (DPT), diphtheria-tetanus (DT), and hepatitis B (Hep B).

TABLE 4

Immunomonitoring of autoimmune markers in serum and CSF of autistic children

Patient code	Blood (serum) ^a			Brain (CSF) ^a		
	Anti-MBP	Anti-MMR	Anti-NAFP	Anti-MBP	Anti-MMR	Anti-NAFP
1. Au063/063	+	+	-	+	-	-
2. Au083/092	+	+	-	+	-	-
3. Au407/408	+	+	-	+	-	-
4. Au284/677	+	+	-	+	+	-
5. Au705/706	+	+	-	+	+	-
6. Au731/721	+	+	-	+	+	-
7. Au739/740	+	+	-	+	-	-
8. Au767/768	+	+	-	+	-	-
9. Au769/770	+	+	+	+	-	-
10. Au771/772	+	+	+	+	-	-
Total (n = 10)	10+	10+	2+	10+	3+	0

Anti-MBP: myelin basic protein autoantibodies; Anti-MMR: measles-mumps-rubella antibodies; Anti-NAFP: neuron-axon filament protein antibodies; CSF: cerebrospinal fluid.

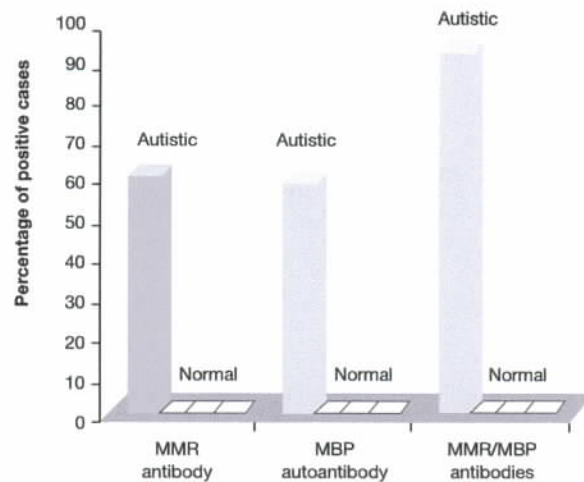
^aThese paired specimens were collected by neurologists of autistic children and sent to the author's laboratory for autoimmune marker analysis. Paired specimens refer to serum and CSF specimens from the same donor procured simultaneously. Immunoblotting was used to detect the presence (+) or absence (-) of these markers.

The laboratory testing revealed that the autistic children had a hyperimmune reaction to MMR vaccine but not to the other 3 vaccines that we investigated (TABLE 3). Extensive characterization by immunoblotting technique showed that the MMR antibodies were specifically directed toward the measles subunit of the MMR vaccine but not against the rubella or mumps subunits.²²⁻²⁴ Furthermore, the immunochemical characterization showed that the immune response (MMR antibodies) was directed toward a 78,000 molecular weight protein of the measles subunit.²² This protein closely resembled the hemagglutinin (HA) antigen, which suggests that the inappropriate immune response in autistic children is most likely directed toward the HA protein of measles virus rather than the nucleoprotein (N) or matrix (M) protein.

In addition, by using protein immunoblotting technique, we also carried out antibody testing of 10 paired specimens of serum and cerebrospinal fluid (CSF) from the same donor with autism. The results, summarized in TABLE 4, showed that all 10 serum samples and cor-

FIGURE 3

Correlations between MMR antibodies and MBP autoantibodies in autistic and normal children



MBP: myelin basic protein; MMR: measles-mumps-rubella.

responding CSF specimens were positive for MBP autoantibodies (anti-MBP); all 10 sera and 3 CSF specimens were positive for MMR antibodies (anti-MMR); and only 2 sera were positive for neuron-axon filament protein antibodies (anti-NAFP), but all 10 CSF specimens were negative for anti-NAFP.

The presence of MBP autoantibodies in both the blood and CSF suggests that the autoimmune reaction is also localized in the brains of autistic children. Furthermore, the presence of MMR antibodies in 3 of 10 CSF specimens (TABLE 4) is a highly positive sign of MMR-acquired measles infection in the brain of these autistic children. Unlike the highly select anti-MBP and anti-MMR immune markers, the nonspecific anti-NAFP marker was not found in CSF specimens. Thus, there is a positive correlation between MMR antibodies and MBP autoantibodies in autistic children, suggesting an etiologic link of MMR-derived measles virus to autoimmunity in autism.²²⁻²⁴

The MMR vaccine is well known to contain human albumin as a stabilizing agent and, thus, we also assayed for antibodies against human albumin. The quantitative level of human albumin antibodies in the sera of autistic children was about the same order of magnitude as

it was in normal children. Moreover, the detection of human albumin antibodies by immunoblotting was mainly a negative result, and it was indistinguishable between autistic children and normal children.²⁴ Thus, the elevated MMR antibodies in autistic children are directed toward the measles subunit of the trivalent vaccine and not against the human albumin, which as stated, is used as a stabilizing protein in the MMR vaccine. Moreover, similar to measles virus alone, we found a strong serologic correlation (>90%) between MMR antibodies and MBP autoantibodies (FIGURE 3).

Collectively, these findings suggested an etiologic link between the MMR vaccine and autoimmunity in autism. As far as we know, this is the first study of its kind to examine associations between a viral factor (virus serology) and an autoimmune factor (brain autoantibodies) in a medical condition (autism/ASD) in which autoimmunity appears to be the core of the problem. Evidently, our study might also represent a novel mechanism by which the so-called autistic regression post-MMR vaccination might be explained in at least some children with ASD.²²

In this respect, although more research must be done, we invoked the hypothesis that an atypical measles infection may be etiologically linked to brain autoimmunity in autism. There is considerable credence to this hypothesis based on studies of autoimmunity-producing cytokines that have been reported in the literature. First, autistic children have significant increases in autoimmunity-inducing cytokines such as interleukin-12 (IL-12) and interferon- γ (IFN- γ) in favor of a Th1 immune response.^{3,38} Second, the measles vaccination with MMR vaccine mainly induces IFN- γ for a Th1 type of immune response. Since the MMR-induced immune response and autoimmune autism involve a common immune cell (Th1), it is quite conceivable that MMR vaccine might also be involved in the pathogenesis of autism.^{22,23}

Taken together, these observations are directly related to the understanding of a basic mechanism of autoimmunity in autism, but we believe more laboratory research must be done to understand their precise role in the pathogenesis of the disorder.

Heavy metal (mercury)-induced markers in autism. The exposure to heavy metals, in particular mercury, is well known to cause immunotoxicity, autoimmunity, and neurotoxicity in genetically predisposed laboratory animals. As cited elsewhere,^{42,43} heavy metal

immunotoxicity is often demonstrated in cell cultures of peripheral blood mononuclear cells in vitro. Consequently, a connection between vaccine-derived mercury (thimerosal) and autism/ASD has been suggested,⁴⁸ albeit with a paucity of laboratory data. Although there are several potential sources of mercury exposure, human exposure to mercury occurs primarily from vaccines because vaccinations or immunizations are mandated by the federal government.

Vaccines contain very small amounts of the chemical thimerosal, which is used as a preservative; however, the MMR vaccine does not contain this preservative, according to the manufacturer. Relying on theoretical grounds, it has been suggested that the mercury-induced neurotoxicologic changes in laboratory animals resemble neurodevelopmental delays in autistic children.⁴⁸ However, this resemblance was not found when neurologists carefully evaluated this hypothetical analogy.⁴⁹ While the controversy continued, we became interested in exploring if autoimmunity in autism could be caused by exposure to mercury. Accordingly, we hypothesized that if autism involves a connection between mercury and autoimmunity, autistic children should harbor elevated levels of mercury-induced autoimmune markers, namely antinucleolar antibodies (ANoA), anti-laminin antibodies (anti-LA), and metallothionein antibodies (anti-MT). In addition, we also studied serum levels of metallothionein (MT) protein, a reliable marker of biological response to mercury and other heavy metals.

We performed laboratory analyses of these markers in autistic children and normal children. The outcome of these laboratory studies was that the distribution of 3 autoimmune markers (ANoA, anti-LA, and anti-MT) and 1 biomarker (MT protein) did not significantly change between autistic children and normal children.^{24,42,43} These were quite clear-cut results of laboratory measurements of highly select markers of mercury exposure. Therefore, based on our laboratory findings, we concluded that mercury is not a critical factor in causing autoimmunity in autism.^{24,42,43} This negative finding, however, does not entirely rule out the possibility that mercury, if it is involved in autism, might trigger some other biochemical event, for example, mitochondrial oxidative stress and/or an inflammatory response.²⁰

POTENTIAL SOURCE OF MEASLES VIRUS IN AUTISM. At present, the source of measles virus in autistic children is not clearly defined. As stated above, the medical history of children with an ASD does not show any record

TABLE 5

Similarities between measles infection and autism

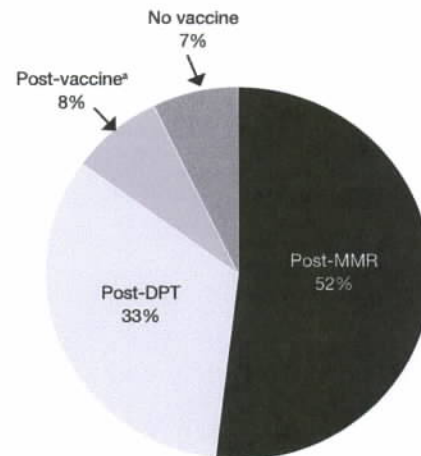
Measles virus (MV)	Autism
1. MV infects brain regions: temporal and frontal lobes of the cerebral cortex, cerebellum, hippocampus, amygdala, cingulated gyrus, and hypothalamus. ⁵⁰	1. Affected brain regions: temporal and frontal lobes of the cerebral cortex, cerebellum, hippocampus, amygdala, cingulated gyrus, and hypothalamus. ⁵¹
2. MV causes immunosuppression, specifically of T helper (CD4+) cell function. ^{52,53}	2. Autistic children have immunosuppression, particularly T helper (CD4+) cell function. ^{32,36,37}
3. MV (MMR-HA) stimulates antigen-presenting cells to produce IFN- γ for Th1 cellular immune response. ^{54,55}	3. Autistic children have elevated levels of IFN- γ and IL-12 for Th1 cellular immune response. ^{3,38}
4. MV causes immune activation to produce elevated levels of sCD8 antigen. ⁵⁶	4. Autistic children have immune activation as shown by elevated level of sCD8 antigen. ⁹
5. MV infection is treated with vitamin A. ^{57,58}	5. Autistic children respond to vitamin A-containing cod liver oil. ⁵⁹

of a typical rubella rash, which means that a wild-type measles infection is rather unlikely to exist in these children. But there exists a possibility of an atypical or asymptomatic measles infection in the absence of a typical measles rash. Such an infection could occur either by a variant measles infection or it could be acquired from immunization with MMR vaccine. An atypical measles infection in the absence of a rash and unusual neurologic symptoms has been found to suggest the existence of a variant measles virus in humans.^{22,47} The most likely explanation for a connection between autism and measles is that some autistic children might be genetically predisposed to the disorder. Measles or MMR may somehow prompt their immune systems to act in a negative way, while leaving other children unharmed. If measles is a culprit in autism, it may not be the only virus to play a role in causing autoimmunity; other viruses and other vaccines should also be investigated. As described in this article, we studied 6 viruses and 4 vaccines and found that autistic children harbored an abnormal antibody response to measles virus only and/or MMR vaccine only. This is a finding with a very high degree of specificity and selectivity; hence, it must be related to the induction of autoimmunity in autism.

Concerning autism and vaccines, we also paid attention to the unsolicited reports that were sent to us by 152 families shortly after the publication of our virus serology paper.²¹ It should be underscored that we were totally unaware of the existence of this particular information and that families rendered their reports of their

FIGURE 4

Distribution of vaccine-induced autism cases from unsolicited reports



DPT: diphtheria-pertussis-tetanus; MMR: measles-mumps-rubella.
*Not identified.

own choosing, ie, this would be similar to a double-blind study.

When we compiled information from these reports, surprisingly, a very interesting pattern emerged (SEE FIGURE 4). Of those reports, the highest proportion of families (approximately 52%) said that the symptoms of autism began shortly after the MMR vaccination, 33% said the problems started days after the DPT shot, 8% said there was a vaccine connection in their autistic children but they did not know which vaccine was

involved, and 7% said there was no vaccine connection with symptoms of their children.

It is of considerable etiologic significance that autism was prevalent among children who received the MMR vaccine. According to these parents, their children were born normal and were developing normally, but there was a sharp regression or developmental delay shortly after the administration of MMR vaccine. Although these reports are nonscientific and unacceptable on scientific grounds, they certainly prompted us to examine vaccines and autism through laboratory research. Thus, we conducted laboratory studies of virus and vaccine serology and brain autoantibodies in children with autism.

As described here, there is a clear-cut serologic association between the MMR-derived measles strain and autism. Although more experimental research is needed to confirm this link, we also noted certain important similarities of manifestations between measles infection and autism/ASD (TABLE 5). Measles infects brain regions such as the temporal and frontal lobes, hippocampus, and amygdala,⁵⁰ which are also affected in the brains of autistic children.⁵¹ Measles virus is well known to induce immunosuppression of T helper cells,^{52,53} whose function is also reduced in children with autism.^{32,34,36,37} The MMR vaccine elicits cellular immunity via Th1 cells^{54,55}; the same type of cell is also involved in autism.^{3,38} Measles virus causes immune activation as reflected by elevated levels of soluble CD8 antigen,⁵⁶ which is also elevated in autistic children.⁹ Furthermore, vitamin A treatment has been used for both measles infection^{57,58} and autistic children.⁵⁹ This resemblance between measles infection and autism, together with our findings of elevated measles serology and abnormal measles antibody response, might point to an etiologic link of measles virus in AAD, as described here.

Cytokine studies in autism

Several years ago, we studied cytokine regulation in autism.^{3,9,38} Cytokine studies can be performed by 3 different approaches: (1) cytokines can be measured in biological fluids such as serum, plasma, or cerebrospinal fluid, which represent endogenously (or in vivo) produced circulating cytokines, (2) cytokine production can be studied by peripheral blood mononuclear cells (PBMNCs) after mitogen stimulation in vitro, and (3) cytokine-specific mRNA expression can be measured in PBMNCs after mitogen stimulation.

We took the first approach because it represents a physiologic state in the body, and so we measured serum levels of cytokines in autistic children. We found that the serum level of only 3 cytokines (IL-2, IL-12, and IFN- γ) was significantly elevated in autistic children³⁸ but the serum levels of 6 other cytokines (IL-1, IL-4, IL-6, IL-10, IFN- α , and TNF- α) did not significantly differ between normal children and autistic children.^{3,9,38} Because of a specific increase of IL-12 and IFN- γ , we were the first to suggest that autism involves the Th1 type of immune response.³⁸ Subsequently, we conducted a study of IL-2, IL-6, and TNF production by PBMNCs.

We found that IL-2 production was significantly increased in autistic children. The production of IL-6 and TNF by PBMNCs of autistic children was moderately higher in autistic children than in normal children, but the difference did not attain statistical significance.³ Our result of TNF production in autistic children is consistent with a previous report.³² Recently, 2 other groups of researchers took alternative approaches and found that PBMNCs of autistic children produce elevated levels of IL-12 and IFN- γ or express higher than normal levels of messenger RNA for IFN- γ (see Singh 2003³). Taken together, these findings demonstrate the existence of the Th1 type of immune response in autistic children,³ which would also be consistent with autoimmune pathology in autism³⁸ because the IL-2, IL-12, and IFN- γ cytokines are well-known inducers of autoimmune diseases.^{60,61}

Regarding the pathogenesis of immune-mediated diseases, immune activation is one of the primary events in autoimmunity, inflammation, and viral infections. Immune activation leads to spontaneous proliferation of PBMNCs, increased expression of activation markers on PBMNCs, and increased accumulation of blood mononuclear cell-derived soluble antigens, mainly cytokines, cytokine receptors, and adhesion molecules.

Based on these considerations, immune activation occurs naturally in autistic children because they have elevated levels of immune-activation antigens such as soluble CD8, IL-2, IL-12, and IFN- γ .^{9,38} and their blood contains activated T cells.^{35,36} Thus, it is reasonable to conclude that the increase of IL-12 in autistic children points to antigenic stimulation of Th1 cells that, via IFN- γ , may induce autoimmunity.^{3,38} The IL-12 cytokine selectively promotes the development of Th1 cells,⁶⁰ and Th1 cells initiate the pathogenesis of organ-specific

autoimmune diseases.⁶¹ Immune activation is also well known to exist in autistic children because they harbor elevated levels of acute-phase reactants, including C-reactive protein (CRP) and S-100 protein,²⁰ that have also been linked to autoimmune diseases.⁶²

Testing for autoimmunity in autism

Deriving from neuroimmunologic research,¹ autoimmunity has been shown to play a key role in the pathogenesis of neurologic disorders.^{9,10} The list of these disorders now also includes autism.²⁻⁴ Since the brain is the affected organ, the autoimmune response will be directed toward the brain.

Autoimmunity is commonly characterized by the expression of certain autoimmune factors. These factors are important for identifying a brain-specific autoimmune response, which we have identified in children with autism. By performing blood tests, we can determine if a patient shows autoimmunity to brain, if he or she is a candidate for experimental immunomodulation therapy, and if the response to therapy is effective. Thus, this type of immune evaluation is extremely important in helping patients with autism. The specific tests are listed below.

Immune panel profile. It is strongly recommended that all children with autism/ASD be tested for a basic immune panel profile. This panel includes testing them for serum immunoglobulins; a complete blood count for mononuclear cells (lymphocytes and monocytes); blood enumeration of T cells, B cells, NK cells, and T-cell subsets (helper T cells and suppressor T cells). For research purposes, it is also important to evaluate lymphocyte function, for example, mitogen-stimulated lymphocyte proliferation and NK cell activity. All of these immune parameters have been found to be abnormal in children with autism/ASD (SEE TABLE 1 and Singh⁴).

Brain autoantibody profile. This test detects antibodies to 2 brain proteins—the MBP and the NAEP. We have found that the incidence of MBP autoantibody in the autistic population is markedly higher than that of the normal population; hence, it serves as a primary marker of the autoimmune reaction in autism. In contrast, the incidence of NAEP antibody in autistic patients is only marginally higher than that of normal controls, making it a secondary marker of choice. It is, however, recommended that these 2 autoimmune markers be tested simultaneously.^{2,10,17,18}

Virus serology profile. This test measures the level

of antibodies to viruses such as measles virus, mumps virus, rubella virus, CMV, or HHV-6. We have shown that the level of measles antibody is elevated in many autistic children, which could be a sign of a present infection, a past infection, or an immune reaction to MMR vaccine.^{2,22,23}

Vaccine serology profile. This test detects antibodies to vaccines, including MMR and DPT. We showed that a significant number of autistic children, but not normal children, harbor a unique type of measles antibody to MMR vaccine. This antibody might represent an abnormal or inappropriate immune reaction to this vaccine and should be tested in relation to autoimmunity in autism.^{2,22}

Cytokine profile. Two cytokines, IL-12 and IFN- γ , play a very important pathogenic role in autoimmune diseases in that they initiate an autoimmune reaction via induction of the Th1 type of white blood cells. We have found that these 2 cytokines are selectively elevated in autistic children, suggesting the induction of autoimmunity via Th1 cells in autism. Therefore, they should be measured as a sign of impaired cellular autoimmunity in patients with autism.^{3,9,38}

Serotonin profile. This test measures the serum or plasma level of serotonin. We have found that patients with autism have abnormal levels of serotonin, which should be tested before administering treatment with selective serotonin reuptake inhibitor (SSRI) therapy. Elevated serotonin levels in autism might also be related to autoimmune reaction to serotonin receptors in the brain.¹⁹

Mercury-induced autoimmune markers. This test assays for autoimmune reaction to mercury (or heavy metal) exposure. These markers include: (1) antinuclear antibodies against nucleolar antigens (ANoA), (2) anti-laminin antibodies (ALA) against basement-membrane proteins, and (3) antibodies to metallothionein protein (anti-MT). In addition, the serum level of MT protein should also be measured as an index of biological response to exposure to heavy metal or mercury. We have found that only a very small number of autistic children are positive for these antibodies and MT protein, but their levels did not differ significantly from normal children.^{42,43}

Acute-phase reaction (APR) markers. This test should be done to assess acute-phase reaction (APR), which is an excellent sign of inflammation. The test includes measurement of certain biomarkers, chiefly C-reactive protein

(CRP) and S100 protein. We recently found significantly higher than normal levels of these 2 markers, suggesting the existence of APR in autistic children.²⁰

Immunomodulation therapy (IMT) in autism

Several lines of laboratory findings have demonstrated the role of autoimmunity in the pathogenesis of autism. The idea that autism is an autoimmune disorder is also strengthened by the fact that autistic patients respond well to treatment with immunomodulating agents.²⁻⁴ Immune intervention can produce immunomodulation—a state of balance between immunosuppression and immunostimulation. Since autistic patients do not show a classical primary immunodeficiency, simply boosting their immunity is not a good strategy. They do, however, have immune abnormalities. Therefore, depending on the nature of the immune abnormality, the goal of immunomodulation therapy (IMT) should be to achieve immune balance by normalizing or reconstituting immune functions. This will allow a more balanced immune response, avoiding major fluctuations of overt immune activity, which could be detrimental to the patient. IMT should always be given in consultation with a physician, preferably a clinical immunologist, allergist, or hematologist. The following is a partial list of IMTs that should be considered for patients with autism.

Transfer factor therapy. Transfer factor (TF) is an immunomodulator for regulating cellular immune functions of NK cells and T lymphocytes, especially during viral and microbial infections. To be effective, TF—commonly known as dialyzable leukocyte extract (DLE)—is normally made from the leukocytes of highly select blood donors. By using DLE-TF, open-label studies have shown symptomatic improvement of some autistic children.^{8,39} Unfortunately, DLE-TF is extremely difficult to prepare, and there are batch-to-batch variations, among other problems inherently associated with the preparation of this particular form of TF. However, there is now a commercial brand of TF (4Life Research, Inc.) that targets NK cells in particular, and that also brings about overall immunomodulation through T lymphocytes in the body.

Autistic children are well known to have faulty immunomodulation, including reduced numbers and function of NK cells, which makes these children good candidates for TF therapy. In our case studies,⁴¹ we recently found that autistic children respond favorably to a scientifically

developed beverage formulation of TF called RioVida (4Life Research, Inc.). To that end, however, the dose of TF should be increased to 1200 to 1800 mg TF per day instead of the recommended daily dose.

As recorded by parents, we observed noticeable improvement in areas of language, speech, social interaction, sleep, attention span, and cognitive behaviors, plus overall better physical health, perhaps due to reduced infections.⁴¹ Furthermore, the improvement in autistic characteristics was also reflected by considerable lowering of the Autism Treatment Evaluation Checklist (ATEC) score (Autism Research Institute, San Diego, CA, USA). Although these findings are preliminary, we think that a properly designed large-scale trial would be an important step in the right direction to help children with autism/ASD with TF-induced immunotherapy.⁴¹

Immunoglobulin therapy. This approach to treatment is already in practice to help autistic children with autoimmune abnormalities. Open-label trials of intravenous immunoglobulin (IV-IG) have shown that most, but not all, autistic children respond favorably to this treatment.³² Clinically, children so treated have shown improvements in language, communication, social interaction, and attention span.

Several years ago, we suggested the use of “oral-IG” as an alternative approach to IV-IG. In a subjective study, oral-IG showed significant improvement of autistic characteristics in patients with autism/ASD, and the outcome appeared to be about the same as or somewhat better than IV-IG.⁴⁰ Further studies are needed to establish the efficacy of this modality.

Autoantigen therapy. Patients with autoimmune diseases are also treated with oral administration of autoantigens. This is also applicable to autism. Since MBP is the autoantigen in autism, autistic patients have responded positively to nutritional supplements containing brain MBP or brain myelin, for example, Sphingolin.²

Glutathione therapy. Glutathione is a natural immunomodulator, antioxidant, and detoxifier. Owing to these biological functions, glutathione is commonly regarded as the body’s most potent protector against infections, autoimmune problems, and other abnormalities, including oxidative stress.⁶³ Early results show signs of some improvement in autistic children.

Glyconutrient therapy. Recently, glycobiology research has identified certain glyconutrients that con-

tain specialized carbohydrates. One such glyconutrient is Ambrotose (Mannatech, Inc., Coppell, TX, USA). Although scientific evidence is lacking, Ambrotose-containing products have been claimed to improve some behavior in autistic children (see www.mannatech.com).

Steroid therapy. Steroids such as prednisone and/or adrenocorticotropic hormone (ACTH) are commonly used as the first course of treatment for patients with autoimmune diseases. Although clinical trials of steroids in autistic patients have not been performed, there are some reports showing benefits and improvement of behavioral characteristics in patients with autism/ASD.⁶⁴ However, the individual case history of viral infection should be taken into account before administering steroid treatment because viruses can sometimes exert immunosuppressive effects.

Plasmapheresis therapy. Plasmapheresis or plasma exchange (PE) therapy is often used to help patients with infections, autoimmune diseases, and immune complex diseases. PE therapy has been successfully used to improve clinical symptoms in patients with a wide variety of neurologic disorders, including CNS dysmyelination⁶⁵ and obsessive-compulsive disorder.⁶⁶ Because autism involves viral infection and CNS-myelin autoimmunity, we were the first to suggest that this treatment modality be explored in patients with autism.^{2,4}

CONCLUSION

Current scientific research from laboratories worldwide has demonstrated that autoimmunity is the core of the problem in a vast majority of people affected with autism/ASD.^{2-4,41} We have identified and characterized the autoimmune subset as a major subset of autism and designated it as an AAD.⁶⁷ The phenotypic expression of AAD was reflected by the existence of hyperimmune measles/MMR serology, faulty immune regulation, functional imbalance of T lymphocytes and NK cells, brain-specific autoantibodies with MBP as the primary autoantigen, and responsiveness to IMT.

Based on our laboratory data on virus and vaccine serology, we suggest that AAD is likely triggered by a virus, and that measles virus (MV and/or MMR vaccine) might be a very good candidate; however, more experimental research is needed to firmly establish the pathogenic link of measles virus with this brain disorder. In contrast, laboratory analysis of autoimmune markers

did not support the idea that thimerosal-derived mercury induces autoimmunity in autism. The existence of autoimmune problems in autistic patients and their responsiveness to treatment with IMT also supports an etiopathogenic role of virus-induced autoimmunity in autism.

Considering that autism affects an estimated 2 to 2.5 million Americans, if 75% of these individuals have AAD or autoimmunity, a very significant number (1.5 to 1.9 million) could benefit directly from autoimmunity research. If the world population of persons with autism/ASD is taken into account, the impact of autoimmunity research could be much greater, impacting the lives of millions more worldwide.

Therefore, the author of this review article suggests that autism be considered on medical grounds as an autoimmune disorder, which in turn would draw much wider international attention of medical doctors and biomedical researchers. After all, the clinical presentation of autoimmunity is a medical condition and, likewise, autism should also be regarded as a medical condition. In this respect, autism may very well be a psychiatric condition that defines the academic role of psychoneuroimmunology (PNI) or immunopsychiatry in the clinical practice of psychiatry. Naturally, this topic offers a novel direction of future research for helping persons diagnosed with autism and related neurobehavioral disorders.

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