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Review

Autistic disorder and viral infections

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Autistic disorder (autism) is a behaviorally defined developmental disorder with a wide range of behaviors. Although the etiology of autism is unknown, data suggest that autism results from multiple etiologies with both genetic and environmental contributions, which may explain the spectrum of behaviors seen in this disorder. One proposed etiology for autism is viral infection very early in development. The mechanism, by which viral infection may lead to autism, be it through direct infection of the central nervous system (CNS), through infection elsewhere in the body acting as a trigger for disease in the CNS, through alteration of the immune response of the mother or offspring, or through a combination of these, is not yet known. Animal models in which early viral infection results in behavioral changes later in life include the influenza virus model in pregnant mice and the Borna disease virus model in newborn Lewis rats. Many studies over the years have presented evidence both for and against the association of autism with various viral infections. The best association to date has been made between congenital rubella and autism; however, members of the herpes virus family may also have a role in autism. Recently, controversy has arisen as to the involvement of measles virus and/or the measles, mumps, rubella (MMR) vaccine in the development of autism. Biological assays lend support to the association between measles virus or MMR and autism whereas epidemiologic studies show no association between MMR and autism. Further research is needed to clarify both the mechanisms whereby viral infection early in development may lead to autism and the possible involvement of the MMR vaccine in the development of autism. *Journal of NeuroVirology* (2005) **11,** 1–10.

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Introduction

Autistic disorder (autism) is a complex developmental syndrome of the central nervous system (CNS) that is the prototype for a group of disorders known as a pervasive developmental disorder (PDD). Autism is behaviorally defined, manifesting with deficits in communication and social interactions in combination with stereotyped or restricted behaviors or interests. Onset is prior to 3 years of age (American Psychiatric Association, 1994). Autistic regression is the sudden or gradual deterioration leading to an autistic state of a previously developmentally normal child (Tuchman and Rapin, 1997). The wide ranges of autistic behaviors are often referred to as autistic spectrum disorder (ASD). Early epidemiological studies reported that autism occurred at a rate of approximately 4 to 5 per 10,000 live births and at a ratio of 4:1, boys to girls (Ciaranello and Ciaranello, 1995; Fombonne, 1999). More recent studies find a higher prevalence (i.e., 3.4 per 1000 [Yeargin-Allsopp *et al*, 2003]; 1.6 per 1000 [Chakrabarti and Fombonne, 2001]). Whether this increase is due to changing diagnostic practices or actual increased incidence is a matter of debate.

Despite decades of research, very little has been learned about the etiology and/or pathophysiology of autism. Thus far, data suggest that autism is

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most likely the result of multiple etiologies with genetic and environmental contributions (Coleman and Gillberg, 1985; Nelson, 1991). Neuropathology can vary between cases (Sweeten *et al*, 2002); however, the most consistent pathology includes curtailment of normal development in the limbic system and abnormal development of the cerebellum and associated nuclei (Kemper and Bauman, 1998; Ritvo *et al*, 1986).

One prominent factor contributing to the development of autism is a genetic predisposition (Bailey *et al*, 1995). It has been reported that the risk of developing autism for subsequent siblings of an autistic child is 3% to 7%, or a 50-fold increase over families without an autistic child. Also, if one monozygotic (identical) twin develops autism, the risk to the second twin is approximately 36% to 91% (Coleman and Gillberg, 1985; Wong, 2001; Bailey *et al*, 1995; Steffenburg *et al*, 1989). The risk of this second monozygotic twin developing a condition within ASD is 92% (Bailey *et al*, 1995; Wong, 2001). In comparison, if one dizygotic (nonidentical) twin develops autism, the risk to the second twin is virtually 0% for developing autism and 10% for developing a condition within ASD (Wong, 2001; Bailey *et al*, 1995). The great difference in concordance between monozygotic and dizygotic twin pairs suggests that multiple genes are involved. In spite of strong family and twin study evidence for genetic factors, genome scans have failed to detect loci linked to ASD or PDD; these studies indicate that as many as 20 genes may be involved (Cook, 2001; Risch *et al*, 1999). In addition, the monozygotic concordance of less than 100% suggests that environmental factors may contribute to etiology.

Virus/cytokine hypothesis and autism

One proposed etiology for autism is prenatal or early infantile viral infections. The CNS is not fully developed at birth (Rosenberger, 1975) and is more susceptible to viral damage (Sells *et al*, 1975). Resistance to CNS virus infections increases with age. For example, herpes viruses and other viruses can readily cause encephalitis in newborn animals, but as the animal matures it becomes resistant (Johnson, 1982). The outcome of exposure to prenatal viral infection depends on many factors, including the maternal immune status, the infecting virus, the strain of virus, the variation in susceptibility of the maternal and fetal host, the developmental stage of the fetus, the amount of virus reaching the fetus—particularly the CNS and immune system, the route of access, genetics, and probably other factors (Blattner, 1974).

Upon viral infection, the immune response leads to the production of various cytokines. Through receptor-mediated events, cytokines substantially affect immune and CNS cells. For instance, cytokines such as interleukin (IL)-1, -2, and -6 have been shown to alter neuronal release of dopamine, acetylcholine, serotonin, and norepinephrine in the hippocampus and other brain regions (Zalcman *et al*, 1994; Araujo *et al*, 1989; Hanisch *et al*, 1993).

Pregnancy is immunosuppressive and women become more susceptible to infections (Weinberg, 1984; Sargent, 1993). Expectant mothers often report having colds or infections during pregnancy. We do not know whether infection of the mother or offspring could initiate events in a genetically susceptible individual that leads to autism. An acute infection could lead to transient levels of cytokines without viral persistence, or infection could instigate an autoimmune process resulting in chronically elevated cytokine production. A persistent viral infection could also lead to chronically elevated cytokine levels. Depending on the location of immune activation, cytokines could be produced directly in the brain or gain access to the CNS by crossing an immature blood-brain barrier (BBB). Abnormal brain cytokine levels can alter CNS development (Muñoz-Fernández and Fresno, 1998). For example, transgenic mice with astrocyte-directed expression of interferon (IFN)-γ develop severe cerebellar and hippocampal dysplasia (LaFerla *et al*, 2000), and in cultures of rat embryonic hippocampal neurons IFN-γ inhibits dendritic outgrowths, decreasing synaptic formation (Kim *et al*, 2002). Reduced complexity of dendritic branching has been shown in the hippocampus of autistic brains (Raymond *et al*, 1996).

A mouse model has been developed to investigate viral infection of pregnant mothers (Patterson, 2002; Shi *et al*, 2003). Respiratory infection with the human influenza virus at mid-gestation results in behavioral and pharmacological abnormalities, indicating an effect on fetal brain development caused by the maternal antiviral immune response (cytokines). Cytokine changes have not yet been measured in the fetal brain in the influenza model; therefore, it is not yet known whether the cytokines are the product of the fetus or the mother. These behavioral changes remain into adulthood in the case of both BALB/c and C57BL/6 mice. The results are highly significant changes in several behaviors, not specifically analogous to autism (Shi *et al*, 2003). An antiviral immune response can be induced without infection by intraperitoneal injection of polyinosinic-polycytidylic acid (Patterson, 2002; Shi *et al*, 2003). This injection also results in behavioral abnormalities similar to the infection model. This mouse model can be used to investigate which components of the immune response cause changes in the fetal brain that results in behavioral changes.

Some parallels to autism have been observed in Lewis rats inoculated intracerebrally with Borna disease virus shortly after birth (Hornig *et al*, 1999). Behaviorally these rats displayed stereotypic behaviors,

dysregulated exploratory activity, growth delay, aberrant righting reflexes, and asymmetries in motor responses. Neuronal apoptosis occurred predominately in the hippocampus and cerebellum. Increased mRNA transcripts for IL-1α, -1β and -6 and tumor necrosis factor-α were observed in multiple brain regions in infected rats (Hornig *et al*, 1999). Other researchers have shown that Lewis rats neonatally infected with Borna disease virus demonstrate deficits in play behavior and other social interaction (Pletnikov *et al*, 1999). Although blood studies of children with autism do not show evidence of Borna disease virus infection (Hornig *et al*, 1999), these infection models of developing animals provide some evidence that viral infections and the ensuing immune response can disrupt neurodevelopment and promote behavioral abnormalities with some similarities to autism.

Virus association with ASD

Many studies have attempted to associate autism with various viral infections (Table 1). Examples of studies finding no association include Anlar *et al* (1994) who found no association between intrauterine human parvovirus infection and infantile autism, and Jorgensen *et al* (1982) who found no association between herpes simplex virus (HSV) serum antibody levels and autism. Another study finding no relationship between individual virus infections was performed by Deykin and MacMahon (1979). They imply that prenatal or early infantile exposure to measles, rubella, mumps, or chickenpox was unlikely to play a major role in a substantial proportion of autism cases. Interestingly, however, they suggest that an "increased frequency of combined illness and exposure was seen for the (autistic) cases relative to their sibling controls to prenatal measles, rubella, mumps and to postnatal mumps" (Deykin and MacMahon, 1979).

In contrast, other studies have found connections between various viral infections and the development of autism (Table 1). Gregg (1941) first linked congenital rubella virus infection to defects in the newborn, and Desmond *et al* (1970) put forward the hypothesis that congenital rubella virus infection could contribute to autism. Stubbs (1976) examined the relationship between prenatal rubella and autism and found that autistic children had an altered immune response to rubella vaccination indicating that they had been congenitally infected with rubella virus. Chess (1971, 1977) conducted studies linking congenital rubella to autism. Chess (1971) found that children who had congenital rubella had an increased incidence of autism. The prevalence rates were 412 children with the complete syndrome of autism (Kanner's classical criteria) plus 329 children with a partial autism syndrome (displaying a significant number of signs of autistic behavior) for a total of 741 children with autism per 10,000 children having congenital rubella. This is in comparison to two other studies, which found prevalence rates of 2.1 children with autism per 10,000 child population (Lotter, 1966) and 0.7 children with autism per 10,000 child population (Treffert, 1970). From these data, Chess proposed that in some children congenital viral infections of the CNS could produce the severe and complex symptoms of autism (Chess, 1977).

Additional viruses have also been reported to be associated with autism. Most of these reports are case studies linking viruses such as herpes simplex, cytomegalovirus (CMV), varicella, and mumps to autism (Ciaranello and Ciaranello, 1995). Some of these reports suggest that HSV encephalitis can trigger autism. DeLong *et al* (1981) first described three children, who developed autism after unknown encephalopathic illness or, in one instance, HSV encephalitis. These children, older than those usually diagnosed with autism, were between the ages of 5 and 11. Interestingly, as the encephalitis abated some of the features of autism did so as well. In contrast, Gillberg (1986) described the development of the classical symptoms of autism over a 70-day period following herpes encephalitis in a 14-year-old girl. This was followed by a report by Greer *et al* (1989) who described the development of autism in another older child, a 14-year-old male, whose autistic symptoms continued after the acute signs of herpes

Table 1 Virus association with autism

No association	*References*	*Association*	*References*
Measles	Deykin and MacMahon, 1979	Measles	Singh *et al*, 1998, 2002; Singh and Jensen, 2003; Uhlmann *et al*, 2002
Rubella	Deykin and MacMahon, 1979	Congenital rubella	Gregg, 1941; Desmond *et al*, 1970; Stubbs, 1976; Chess, 1971, 1977
Herpes simplex virus	Jorgensen *et al*, 1982	Herpes simplex virus	DeLong *et al*, 1981; Gillberg, 1986; Greer *et al*, 1989; Ghaziuddin *et al*, 1992
Mumps	Deykin and MacMahon, 1979	Mumps	Ciaranello and Ciaranello, 1995
Varicella (chicken pox)	Deykin and MacMahon, 1979	Varicella	Knobloch and Pasamanick, 1975
Intrauterine human parvovirus	Anlar *et al*, 1994	Cytomegalovirus (CMV)	Stubbs, 1978; Markowitz, 1983; Ivarsson *et al*, 1990; Sweeten *et al*, 2004a
—	—	Stealth virus	Martin, 1995

encephalitis had diminished. Ghaziuddin *et al* (1992) documented two patients who in all likelihood were infected *in utero* or soon after birth with HSV and developed herpes encephalitis. Both children developed autism as defined in the *Diagnostic and Statistical Manual of Mental Disorders* (Third Edition—Revised) (DSM-III-R) prior to age three.

Many encephalitic patients had temporal lobe involvement (DeLong *et al*, 1981; Gillberg, 1986; Ghaziuddin *et al*, 1992). Hetzler and Griffin (1981) have provided support for the hypothesis that autism results from neuropathology relating to the temporal lobes. They speculate the differences in the extent and neuropathologies of the temporal lobes contribute to the heterogeneity of autism. A similar inference was made by Fotheringham (reviewed in Fotheringham, 1991).

Several case reports have also linked congenital CMV infection (another herpes virus family member) and autism (Stubbs, 1978; Markowitz, 1983; Ivarsson *et al*, 1990; Sweeten *et al*, 2004a). One of the CMV-infected patients who showed an onset prior to age 2 had some improvement but still retained marked deviant behavior (Markowitz, 1983). Ivarsson *et al* (1990) suggested that maternal infection with CMV led to the congenital infection of two patients who subsequently were diagnosed with autism. Thus, members of the herpes virus family have been implicated as having a role in autism. Herpes viruses can induce a variety of proinflammatory cytokines during infections (Dalod *et al*, 2002; Salazar-Mather *et al*, 2000; Orange and Biron, 1996), along with elevated IFN in the brain during HSV encephalitis (Legaspi *et al*, 1980).

Curiously, there have been numerous reports claiming that there are seasonal differences in the births of children with autism (Bartlik, 1981; Fombonne, 1989; Gillberg, 1990; Konstantareas *et al*, 1986; Tanoue *et al*, 1988). Ticher *et al* (1996) reanalyzed data from England, Japan, and Israel and found major periodicities and minor periodicities in the occurrence of autistic births, and proposed from these data that viral pandemics may play a role in the etiology of autism. Epidemiological studies in Israel find a positive association between the rate of measles and viral meningitis in the general population and the autistic birth pattern (Ring *et al*, 1997). More specifically, the birth of autistic subjects with comorbid epilepsy correlate with the rate of viral meningitis in the general population (Barak *et al*, 1999). However, Landau *et al* found no evidence of seasonality in their attempt at replication (Landau *et al*, 1999).

Others investigators have examined cases of autism associated with postnatal varicella encephalitis (Knobloch and Pasamanick, 1975), and a stealth virus encephalopathy (Martin, 1995). Overall, these observations suggest that infection of the CNS by certain viruses during early or late prenatal development or postnatally could promote the development of PDD in susceptible individuals.

Measles/vaccines and autism

A case for measles virus infection and autism has been made by Singh *et al* (1998). This group tested for the presence of antibodies against measles virus in 48 autistic children between the ages of 4 and 12. The 34 normal controls used in this study were between the ages of 5 and 50; only 19 of the normal controls were between the ages of 5 and 12. Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used to determine viral antibody titers in sera, while autoantibodies to brain were detected by immunoblotting. Eighty-five percent of the autistic samples and 82% of the normal controls had a positive titer for anti–measles virus antibodies, which was not a significant difference. However, nearly 70% of autistic sera had anti–myelin basic protein antibodies and 57% had anti–neuron-axon filament protein, whereas none of the control sera were positive for these autoantibodies. In the autistic subjects a positive association between serology and autoantibodies was indicated (Singh *et al*, 1998). A second study by the same group compared 125 autistic children between the ages of 4 and 10 versus 92 normal controls between the ages of 4 and 13, 28 of which had other behavioral diseases (Singh *et al*, 2002). ELISAs and immunoblotting were used to screen serum antibodies. The ELISAs showed that autistic children had a significantly higher level of anti–measles-mumps-rubella antibodies than normal or other disease children. The immunoblotting showed that 60% of the sera from autistic children had antibodies against the measles hemagglutinin protein as compared to 0% of control sera. No control children, but 56% of the autistic children, had autoantibodies reacting to myelin basic protein. A third study used ELISAs to test individually for serum antibodies against measles, mumps, or rubella (Singh and Jensen, 2003). The results showed that 87 autistic children (ages 3 to 10 years) had a statistically higher amount of anti-measles serum antibodies than 32 normal controls (ages 4 to 10 years) or 14 siblings (ages 40 to 11 years). The serum antibody levels against mumps and rubella were not different between autistic children and normal controls and siblings. Immunoblotting of the serum antibodies to the measles virus vaccine showed that 83% (43 of 52) of the autistic samples were immunopositive for a 74-kDa size band, whereas none of the 30 normal controls nor any of the 15 siblings were immunopositive for any bands (Singh and Jensen, 2003). The authors suggest that an inappropriate or abnormal antibody response to the measles part of the measles, mumps, rubella (MMR) vaccine might be related to autism pathogenesis.

Wakefield and associates (Uhlmann *et al*, 2002; Wakefield *et al*, 1998) have proposed a new form or variant of inflammatory bowel disease, ileocolonic

lymphonodular hyperplasia, in children with developmental disorders (autism). Biopsies from the terminal ileum of 91 affected (gut pathology and developmental disorder) children (ages 3 to 14 years) were tested for the presence of measles virus fusion and hemagglutinin genes via TaqMan reverse transcriptase–polymerase chain reaction (RT-PCR) and nucleocapsid gene via RT *in situ* PCR, as compared to 70 developmentally normal controls (ages 0 to 17 years). Gut tissue was positive for the presence of measles virus in 75 of the 91 affected children, whereas only 5 of 70 controls were positive for measles virus. The measles virus was detected in dendritic cells and mature lymphocytes. Therefore, there appears to be an association between the presence of measles virus in the gut and gut pathology in children with developmental disorder as well as a possible interaction between measles virus and the immune response. The authors suggest that measles virus may be a potential immunological trigger for the gut pathology.

Controversy has arisen as to whether an association between the administration of the MMR vaccine and the development of autism exists. There appears to have been a steady increase in the incidence of autism over the past two decades (Taylor *et al*, 1999; Croen *et al*, 2002; Dales *et al*, 2001), and it has been proposed that this increase is due to the introduction of MMR immunization (Wakefield and Montgomery, 1999). Wakefield and colleagues (Wakefield *et al*, 1998; Wakefield and Montgomery, 1999) originally suggested such an association based on parental reports. In one study either the parents or the physicians of 8 out of 12 children (9 of which had autism) linked the child's behavioral problems with MMR vaccination (Wakefield *et al*, 1998). The interval between the MMR and the first behavioral symptoms was 1 to 14 days, 6.3 days average (Wakefield *et al*, 1998). Wakefield and Montgomery (1999) found that along with the rise in the incidence of autism, there was a decrease in the proportion of autistic children with mental retardation. They proposed that the changing pattern of autism may be due to a change in the pattern of exposure to an environmental trigger, such as a delay in exposure until after the period of normal development of the CNS (Wakefield and Montgomery, 1999), as would occur with vaccination replacing congenital infections.

Further, the proposition has been made that the mixture of the three viruses in the MMR vaccine may compound the risk for autism (Wakefield and Montgomery, 1999). However, studies of MMR vaccine reports have shown that the frequencies of adverse events are the same for MMR as for each vaccine when given separately (reviewed in Halsey and Hyman, 2001). Adverse events include fever and rash for measles, arthralgia and arthritis for rubella, and parotitis for mumps. Also, no increased rate of complications was seen in children who had more than one infection of measles, mumps, or rubella at the same time or during the same season (Halsey and Hyman, 2001). Unlike rubella, measles does not usually cause congenital defects. Instead, either abortion results or the acute disease in the neonate is clinically similar to acute disease in older children (Blattner, 1974); however, measles virus can cause a CNS disease known as subacute sclerosing panencephalitis (SSPE) (Oldstone, 1982).

In support of an association between MMR and autism, Kawashima and colleagues (Kawashima *et al*, 2000) detected measles virus genomic RNA, via RT-PCR, in peripheral blood mononuclear cells (PBMCs) obtained from Wakefield's group from three of nine autistic children (one-third of patients) with regressive autism having gut manifestations, in the age range of 3 to 10 years. The sequence was found to be consistent with the Schwarz vaccine strain of measles virus (Kawashima *et al*, 2000). The behavioral changes in these nine children were reported to have developed soon after MMR administration. Twenty-two healthy controls, two human immunodeficiency virus (HIV)–infected patients, and four patients with systemic lupus erythematosus (SLE) were negative for measles virus genomic RNA. No ages or sexes were reported for the control individuals studied.

Numerous epidemiological studies do not support a causal association between MMR vaccine and autism (Makela *et al*, 2002; Madsen *et al*, 2002; Taylor *et al*, 2002; Fombonne and Chakrabarti, 2001; Dales *et al*, 2001; Kaye *et al*, 2001; Taylor *et al*, 1999; Peltola *et al*, 1998). Taylor *et al* (1999) have refuted the association between MMR and autism based on the lack of a sudden "step-up" in incidence of autism after the introduction of the MMR vaccine. Also, developmental regression does not cluster in the months following MMR vaccination. They believe that the temporal association between MMR vaccination, given between 12 and 15 months of age, and the first parental concerns for autism, seen around 18 to 19 months of age, is by chance alone (Taylor *et al*, 1999). A follow-up study by the same group found that neither bowel problems nor autistic regression was related to MMR vaccination (Taylor *et al*, 2002). A study of autistic boys born between 1988 and 1993 in the United Kingdom found that the incidence of autism increased during the time period while the prevalence of MMR immunization was over 95% and virtually constant over the same time period (Kaye *et al*, 2001). A similar result was observed when comparing autism rates and MMR immunization coverage in children born between 1980 and 1994 in California (Dales *et al*, 2001).

A large retrospective cohort study of all children born in Denmark from January 1991 through December 1998 investigated the potential role of MMR in causing autism (Madsen *et al*, 2002). Information including MMR vaccination status and diagnosis of autism or related disorders was collected on

537,303 children. Eighty-two percent of the children in the cohort received the MMR vaccine. There was no increase in risk of autism or other ASDs among vaccinated children compared to unvaccinated children (Madsen *et al*, 2002). Taken together, these epidemiological studies provide strong evidence against MMR vaccination triggering ASDs.

Discussion

Although the evidence indicates that genetics play a prominent role in the etiology of autism, environmental factors also contribute to the syndrome. In general, genetic background influences an individual's susceptibility to infection and/or autoimmune disease. In these diseases, immune genes such as those found in the human leukocyte-associated antigen (HLA) have an important impact on disease susceptibility or outcome (Thio *et al*, 2003; Wong and Wen, 2003). Some studies (Warren *et al*, 1996; Torres *et al*, 2002) but not all (Rogers *et al*, 1999) provide evidence for HLA involvement in autism. Family history studies have found increased autoimmune disease in families with autistic probands, suggesting involvement of genes that regulate autoimmune disease susceptibility in autism (Comi *et al*, 1999; Sweeten *et al*, 2003a). The increased prevalence of autism in children with congenital rubella and case reports of autism developing after viral infections suggest that in some instances viral infection could be causal for autistic-like behaviors. However, many of these cases are atypical as they are associated with additional deficits (Stubbs, 1978).

The mechanism(s) whereby acute or chronic viral infections could lead to autism are speculative. Perhaps cytokines play a role, as their production is increased during immune activation, and they have profound effects on brain function and neurodevelopment. Evidence for increased cytokine production exists in autistic subjects (Stubbs, 1995; Jyonouchi *et al*, 2001; Gupta *et al*, 1996). Given the tropism of many viruses to brain regions putatively involved in regulating autistic behavior, viral encephalitis could result in direct destruction of brain cells leading to autistic behavior. Another possibility is that infection triggers a pathological autoimmune response (Money *et al*, 1971; Sweeten *et al*, 2004b). Increased autoantibodies to brain proteins and evidence of immune activation have been observed in autistic subjects (Connolly *et al*, 1999; Sweeten *et al*, 2003b). The HLA and family autoimmune history studies previously mentioned (Warren *et al*, 1996; Torres *et al*, 2002; Comi *et al*, 1999; Sweeten *et al*, 2003a) also support this theory, which has been reviewed in greater detail by others (van Gent *et al*, 1997; Krause *et al*, 2002).

The possibility also exists that a mother's immune system could attack a developing fetus and cause abnormal neurodevelopment. In support of this theory, Warren *et al* (1990) found that mothers of autistic children had increased antibody reactivity to lymphocytes of their autistic children; and Dalton *et al* (2003) detected serum antibodies in the mother of an autistic child that bound to rodent Purkinje cells and other neurons. When this mother's serum was injected into pregnant mice, offspring had altered exploration and motor coordination along with changes in cerebellar magnetic resonance spectroscopy compared to controls.

Wakefield *et al* (1998) have suggested that an inflamed or dysfunctional intestine due to chronic measles infection could play a part in behavioral changes in some children by allowing incomplete breakdown and excessive absorption of opioid-like peptides from food. These peptides would either exert direct effects on brain opioid receptors or alter the function of endogenous opioids by interfering with peptidases required for endogenous opioid breakdown. It is unclear how these food-derived peptides would cross the BBB.

Viruses have long been suspected as microbial triggers for immune-mediated diseases such as multiples sclerosis (MS) and inflammatory bowel disease. However, showing a direct role for viral causation in these and related disease states has proved to be difficult. Generations of researchers have implicated over 20 different viruses as causative agents in MS (as reviewed by Enbom, 2001; Fujinami and Libbey, 1999). Patients with MS produce elevated levels of antibodies to viruses such as measles (Adams and Imagawa, 1962), human herpesvirus 6 (Sola *et al*, 1993), and Epstein-Barr virus (Sumaya *et al*, 1985). Evidence exists for the presence of various viruses in MS plaques (Haase *et al*, 1981; Challoner *et al*, 1995; Geeraedts *et al*, 2004), and a mouse model of viral infection with Theiler's murine encephalomyelitis virus is extensively used as a paradigm for MS (Tsunoda and Fujinami, 1999). Yet, definitive evidence that viruses trigger MS does not exist, but association with virus infections remain.

For inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, a similar scenario exists. Numerous viruses and other pathogens have been implicated in its pathogenesis, yet proof of causation is lacking. A relevant example of this involves the detection of measles virus in patients with inflammatory bowel disease. The same research groups that found evidence of measles virus infection in PBMCs and intestines of autistic subjects have also detected measles virus in PBMCs and intestinal tissue of subjects with inflammatory bowel disease (Kawashima *et al*, 2000; Wakefield *et al*, 1993). However, numerous groups have not been able to replicate these findings (as reviewed by Ghosh *et al*, 2001).

The role of measles and MMR vaccination in triggering autism remains controversial despite a large body of epidemiological evidence that does not lend support to this theory. Given that the studies supporting the role for measles/MMR in autism are biological

in nature, further investigation using similar biological techniques in independent laboratories is needed to ultimately clarify this controversial issue. Further investigations could also examine brain tissue or cerebral spinal fluid for evidence of viral infection; and in addition, epidemiological studies could be employed to determine the co-occurrence of congenital or perinatal infections and autism. If research can consistently show evidence of viral infection in a subgroup of subjects with autism, the daunting task would still remain to determine what role, if any, the virus(es) might play in creating autistic behavior.

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