

# IMMUNOLOGICAL FINDINGS IN AUTISM

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Autism is a disorder of neurobiological origin characterized by impairment of contact and communications. Typical symptoms of autism include extreme withdrawal and an abnormal absorption in fantasy, accompanied by delusion, hallucination, and an inability to communicate verbally or to otherwise relate to people. The cause of autism remains unknown. However, there are several factors including infectious, neurological, metabolic, environmental, and immunologic origin that have been thought to be involved in the disease development process of autism. The cellular entities playing a role in the pathologic processes in the autistic brain are the neurons, glial cells, endothelial cells, microglial cells, and astrocytes with blood brain barrier permeability playing an important role for the trafficking of the immune cells and mediators. In this chapter

immunologic findings on autism are discussed. Particular emphasis is made on the aspects of immunological dysfunctions and inflammation as the two important immunological principles contributing to the diseases process in autism.

### I. Introduction

The initial identification of autism dates back to 1943, when Dr. Kanner first observed a syndrome of abnormal neurological development and impaired social interactions, restricted stereotyped interests, and abnormalities in verbal and nonverbal behavior among several children. Kanner called this stereotypic behavioral disorder autism. Since then, the incidence rate in autism has increased and this disorder is currently one of the major pediatric health concerns in the United States. In 1997, the Centers for Disease Control and Prevention (1999) estimated that a broad definition of autism or autistic spectrum disorders (ASD) may be present in as many as one out of every 500 children. Studies in neuroimaging (Minshew *et al.*, 1993), anatomy, and cytotechnology (Bailey *et al.*, 1998a; Bauman and Kemper, 1994), and epidemiologic (Gillberg, 1990) findings suggest that ASD results from a variety of quantitative and qualitative abnormalities in brain structure. Some molecular, genetic, and cellular characteristics have been identified in cell types including the neurons, glial cells, endothelial cells, microglial cells, and astrocytes of the central nervous system.

Symptomatic manifestations of autism occur within the first 5 years of life and persist into adulthood. The neuropathological abnormalities in this disease have been largely confined to the cerebellum and medial temporal structures. Thus, their possible involvement in autistic development has been the subject of much interest. Several investigators reported cerebellar abnormality in autistic samples (Bauman and Kemper, 1994; Courchesne *et al.*, 1988, 1994; Ritvo *et al.*, 1986). However, some of these studies are debatable and need further confirmation (Bailey and Cox, 1996; Bailey *et al.*, 1998b). Furthermore, evidence for a decrease in cerebellar cell size with no differences in Purkinje cell densities between the normal and autistic children has been reported in literature (Fatemi *et al.*, 2002). Studies of Carper and Courchesne (2000), and Bailey and Cox (1998) have demonstrated that the degree of frontal lobe abnormality correlated with the degree of cerebellar abnormality. The frontal lobe appears to have an excess of neural tissue while the cerebellum has too little neuronal cells in autistic patients.

Even though the causes of autism remain debatable, some scientific findings provide further clues. Large/small brain size and volume, asymmetry in the right hemisphere, attention to details, overlooking the whole along with clumsy

behavior, and chronic inflammation in the central nervous system (CNS) are hallmarks of autism. Studies by Courchesne and his colleagues have shown that newborns who later develop autism have a smaller head size at birth but their head size grows rapidly between 1–2 months and 6–24 (Courchesne *et al.*, 2003). In addition, studies by Herbert and colleagues (2003) demonstrated that there is asymmetrical development of the brain's white matter in autistic children. The brain of children with autism seems to grow normally until age 9 months followed by a rapid period of white matter growth between the period 9–24 months (Courchesne *et al.*, 2003). Thus, in autism, there is asymmetrical-brain maldevelopment and potential abnormality/ies either how the brain is processing information or in the ability of the corpus callosum to network the two sides together where the right hemisphere is especially affected. In addition, studies from Just and his colleagues (2004) illustrated an alteration in brain circuitry causes the inability of autistic patients to utilize the right hemisphere of their brain that normally processes structures to recall the alphabet. However, autistic patients have good ability to appreciate details but little or no ability at conceiving the whole picture. This suggests an overconnectivity of local brain networks while long-range brain wiring are under-connected. Moreover, Teitelbaum and his colleagues (2004) showed that due to skewed brain wiring autistic subjects are clumsy and therefore use unusual strategies for locomotion. In conjunction with these reports is the finding of Goldberg (2000), who demonstrated that the parts of the cerebellum that govern the ability to restore balance operate normally in autistic children. Finally, Vargas *et al.* (2005) reported that the brain tissue of people with autism shows signs of chronic inflammation in the same areas that show excessive growth. The inflammation appears to last a lifetime with a characteristic increase in the number of astroglial cells. The brain areas that show hyperproliferation in white matter also show inflammation. There is also evidence for activated microglia in the spinal fluid (Vargas *et al.*, 2005). Thus, in autistic inflammation there is involvement of astroglial and microglial cells in the absence of lymphocyte infiltration or immunoglobulin deposition in the CNS. There is also increased production of pro-inflammatory and anti-inflammatory cytokines such as MCP-1 and TGF $\beta$ -1 by neuroglia (Vargas *et al.*, 2005). All of these findings support a potential role for dysregulated immunoregulatory process and neuroinflammation in the CNS of patients with autism.

## II. Immune Dysfunction in Autism

Substantial evidence suggests that the immune system plays an important role in the pathogenesis of autism (Bock *et al.*, 2002; Gupta, 2000; Wakefield *et al.*, 1998). While the exact mechanism of immune dysfunction in autistic patients

remains undefined, two general possibilities have been outlined. First, there might be a defect in immune regulation that causes hyper- or hypo-activation of the cellular components of the nervous system. This causes a homeostatic imbalance among the immunoregulatory factors in the brain and/or other affected organs such as the gastrointestinal tract. Second, an alternative mechanism of autistic development has been viewed as autoimmune reaction directed toward a specific target molecule in the brain.

#### A. INVOLVEMENT OF NEURONAL MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) IN AUTISM

Class I and Class II major histocompatibility complex were originally thought to be specific to immune cells, but are also expressed by various other cell types in the brain. In fact, certain allelic products of these genes have been thought to be associated with autism (Daniels *et al.*, 1995; Warren *et al.*, 1991, 1992), including the null allele of the C4B gene (located in the class III region of the MHC), the extended haplotype B44-S30- DR4 (the 44 allele of the HLA-B region, the S allele of the BF gene, the 3 allele of C4A, and the null allele of C4B and the DR4 allele) (Daniels *et al.*, 1995; Warren *et al.*, 1992, 1996). It has also been reported that the third hypervariable region (HVR-3) of certain DRb1 alleles has a very strong association with autism (Warren *et al.*, 1996). These observations provide evidence that MHC genes may be involved in autism.

Furthermore, accumulating evidence indicates that neuronal MHC Class I does not simply function in an immune capacity, but is also crucial for normal brain development, neuronal differentiation, synaptic plasticity, and even behavior. The observation that MHC exists not only in injured brain neurons (Neumann *et al.*, 1995, 1997; Wong *et al.*, 1984, 1985), but also in normal uninfected neurons (*i.e.*, *in vivo*) opens up the possibility of it being involved in normal development as well as in diseases as in autism contributing to the pathophysiology of autistic development (Boulanger and Shatz, 2004). Class I molecules are expressed also by neurons that undergo activity-dependent, long-term structural and synaptic modifications including axonal branching and dendritic growth. In the adult hippocampus, MHC is required for normal long-term potentiation (LTP) and long-term depression (LTD), and is thought to be crucial to learning and memory building process (Boulanger, 2001). Thus, there is no room for immune molecules to reduce neural links (Helmuth, 2000).

Experimental studies with mutant mice genetically deficient in class I MHC or for a class I MHC receptor component, CD3 zeta showed an incomplete

refinement of connections between retina and central targets during development (Huh, 2000). In the hippocampus of adult mutants, N-methyl-D-aspartate receptor-dependent long-term potentiation (LTP) is enhanced, long-term depression (LTD) was absent, and specific class I MHC mRNAs were expressed by distinct mosaics of neurons. These results demonstrated an important role for Class I molecules in the activity-dependent remodeling and plasticity of connections in the developing and mature mammalian central nervous system (CNS). These results clearly show that MHC-I molecules are required for proper development in the CNS (Huh, 2000). Since the pattern of MHC expression is very diverse, it could be reasoned that the expression of MHC is directly related to the neuronal activity (Huh, 2000).

## B. IMPAIRED CELL-MEDIATED IMMUNITY

Cell-mediated immunity is impaired in autism. This includes changes in the numbers and functions of macrophages, T cells, B cells, and natural killer cell activity (Gupta 2000; Warren *et al.*, 1986, 1987). In autistic patients who suffered from frequent gastrointestinal symptoms, Wakefield and colleagues demonstrated that CD3(+) cells were significantly increased in affected children compared with developmentally normal non-inflamed control groups ( $p < 0.01$ ) reaching levels similar to inflamed controls (Ashwood *et al.*, 2003).

## C. T-CELL POLARITY IN AUTISM

Helper T-lymphocytes have been shown to differentiate into two mutually regulatory subsets. Th1-like (IL-2, IFN- $\gamma$ ) mediates classical cell-mediated immune responses such as delayed-type hypersensitivity. Th2-like (IL-4, IL-6, and IL-10) cells promote humoral immune responses, in particular the production of IgE and IgG4 (human) or IgG1 (rodents). Over-activity of either cell type can result in a tissue-damaging autoimmune disease. A number of human diseases including asthma and some kidney diseases are thought to be caused by a Th-2 type autoimmune response. A shift occurs from T helper 1(Th1) to T helper 2 (Th2) T cells in autism as evidenced by a decrease in the production of interleukin-2 (IL-2) and gamma interferon(IFN- $\gamma$ ), but there is an increase in the production of IL-4 (37). An imbalance of Th1/Th2 subsets of CD4<sup>+</sup>/CD8<sup>+</sup> T cells towards Th2 may play a role in the pathogenesis of autism involving an autoimmune phenomenon (discussed later) (van Gent *et al.*, 1997).

#### D. IMPAIRED HUMORAL IMMUNITY

A number of studies have documented abnormal humoral responses in autistic individuals. [Warren and collaborators \(1997\)](#) found decreased serum IgA in 8 of 40 (20%) individuals with autism. [Gupta \(2000\)](#) has demonstrated that the immune system within autistics shows a tendency for upper respiratory tract infections, increased allergy and increased gut yeast infection, and the presence of parasites in some cases. He also found a link with serum immunoglobulin in autistics. These children had increased levels of IgM and IgE and low levels of IgA and IgG1 along with low antibody response to protein antigens ([Gupta, 2000](#)). It is also apparent that there is a low response from Th1 as its levels decrease and Th2 increases. It is assumed that if Th1 drops then the gut is open to viral infection and fungal overgrowth. Therefore, low IgA leads to poor gut protection. This results in lymphatic hyperplasia, due to altered self-antigens that may lead to auto-immunity. However, elevated levels of interleukin-12 and interferon-gamma (IFN- $\gamma$ ) are found in autism ([Singh, 1996](#)). It has been postulated by Singh that abnormal production of interleukin-12 (IL-12), a critical Th1 promoting cytokine, may be a compensatory mechanism in the body of autistic patients that leads to defective cell-mediated immunity and augmented humoral responses.

#### E. BRAIN-SPECIFIC ANTIBODIES

Several antibodies reacting to brain tissue have been reported. Approximately 58% (19 of 33) sera of autistic children (less than or equal to 10 years of age) are found to be positive for anti-myelin basic protein (MBP) in autistic patients ([Singh, 1993](#)).

Autistic children, but not normal children, had antibodies to caudate nucleus (49% positive sera) implying that autoimmune reaction to caudate nucleus of the brain region may cause neurological impairments in autistic children ([Singh and Rivas, 2004](#)). The occurrence of autoreactivities to brain tissue in autistic patients may represent the immune system's neuroprotective response to a previous brain injury that may have occurred during neurodevelopment ([Silva et al., 2004](#)). Antibodies against Purkinje cells and gliadin peptides were also observed in autistic patients ([Vojdani et al., 2004](#)). There was further evidence that serum IgG anti-nuclear autoantibodies and IgM anti-brain endothelial cells antibodies were found in the sera of autistic patients ([Connolly et al., 1999](#)). All these reports strengthen the hypothesis that there is an antibody response which cross-reacts with some component in the brain causing dysfunction of the affected area.

### III. Role of Viral Infections in Autistic Development

Given the immunopathogenic features of autism, the development process of this disease is likely to include infection. In fact, it has been shown in neonatal rat infection with Borna disease virus, a neurotropic noncytolytic RNA virus, is associated with marked alterations in the cerebellum, along with reductions in granule and Purkinje cell numbers. In this infectious model, neurons are lost predominantly by apoptosis, by an increase in mRNA levels for pro-apoptotic products (Fas, caspase-1), a decrease in mRNA levels for the anti-apoptotic bcl-x and in situ labeling of fragmented DNA (Hornig *et al.*, 1999). The inflammatory infiltrates that accompany this infection are observed transiently in frontal cortex. Glial activation (microgliosis > astrocytosis) is prominent throughout the brain and persists for several weeks in concert with increased levels of proinflammatory cytokine mRNAs (interleukins 1alpha, 1beta, and 6 and tumor necrosis factor alpha) and progressive hippocampal and cerebellar damage (Hornig *et al.*, 1999).

Maternal exposure to a sublethal intranasal administration of human influenza virus (H1N1) in C57BL/6 mice in Day 9 corresponding to about the second trimester in humans has a very significant effect in the brain development. Prenatal exposure of pregnant mice with H1N1 virus has both short-term and long-lasting deleterious effects on developing brain structure in the progeny. This was evidenced by altered pyramidal and nonpyramidal cell density values, atrophy of pyramidal cells despite normal cell proliferation rate, and final enlargement of brain (Fatemi *et al.*, 2002).

#### A. ASSOCIATION OF MEASLES VIRUS WITH INFLAMMATORY PROCESS IN AUTISM

Maternal infection is a risk factor for many neurodevelopmental disorders, including autism (Ciaranello and Ciaranello, 1995; Patterson, 2002; Pletnikov *et al.*, 2002).

It was reported that 43% of mothers with an autistic child experienced upper respiratory tract, influenza-like, urinary, or vaginal infections during pregnancy compared to only 26% of control mothers (Comi *et al.*, 1999). Studies show that, in rats, maternal exposure to infection alters proinflammatory cytokine levels in the fetal environment, including the brain. It has been proposed that these changes may have a significant impact on the developing brain (Giralt *et al.*, 2002; Urakubo *et al.*, 2001). These observations suggest certain cases of autism may be a sequela of pathogenic infections, especially those of a viral origin (Ciaranello and Ciaranello, 1995; Hornig *et al.*, 2002; Pletnikov *et al.*, 2002).

The target sites for measles virus (MV) are similar to the sites affected by autism. These include the cerebellum, the hippocampus, amygdala, cingulate gyrus, hypothalamus, and the frontal and temporal lobes of the cerebral cortex. Although the route of infection by MV is respiratory, and despite its widespread dissemination to the skin, the intestinal tract and the nervous system are the organs affected. The virus has a strong predilection for lymphoid tissues in the early as well as late stages of the disease.

Human CD46 and CDw150 serve as two receptors for MV induced immunosuppression. CD46 molecule, a member of the complement regulatory cascade of proteins (Dorig *et al.*, 1993; Manchester *et al.*, 1994; Nanche *et al.*, 1993) is ubiquitously expressed on all nucleated cells (McQuaid and Cosby, 2002). CDw150 (signaling lymphocyte activation molecule, or SLAM) is a T-cell costimulatory molecule and is expressed only on immature thymocytes, activated and memory T cells, B cells, activated monocytes, and dendritic cells (Cocks *et al.*, 1995; McQuaid and Cosby, 2002; Minagawa *et al.*, 2001; Punnonen *et al.*, 1997; Sidorenko and Clark, 1993). These two receptors induce marked host immune suppression. Although monocytes express CD46, they are considerably resistant to MV. Once monocytes differentiate into immature myeloid dendritic cells (iDCs) (GM-CSF + IL-4-treated), the cells become susceptible to MV (Murabayashi *et al.*, 2002). DCs that matured via stimulation of their Toll-like receptors (TLRs) 2 and/or 4 exhibited an approximately fivefold increase in CDw150 at the protein level, resulting in higher levels of MV amplification in mixed culture of lymphocytes than in iDCs without TLR2/4 stimuli (Murabayashi *et al.*, 2002).

Measles stimulates maturation of antigen-presenting cells in skin, gut, and lungs. Measles also induces IL-6 from fibroblasts and interferon  $\beta$  and colony-stimulating activity from granulocytes and monocytes such as granulocyte-macrophage colony stimulating factor (GM-CSF) (Van Damme *et al.*, 1989). This consequently requires regulation of the immune system during future infections (Murabayashi *et al.*, 2002). Aberrant expression of TGF $\beta$ 1 can stimulate inflammatory and fibrotic tissue formation and high intracellular TGF $\beta$ 1 may induce over-expression of CD46 receptors, a portal for measles virus entry (Pasch *et al.*, 1999).

At the cellular level, MV causes cell cycle cessation, especially during the GO/G1 phase where major decisions regarding the cell's fate are determined (Schrage *et al.*, 1999). If the CD46 receptor is unavailable (Dorig *et al.*, 1993), then growth factor receptors (e.g., IGF-1 and epidermal growth factor (EGF) receptors) are used for viral entry (Schneider *et al.*, 2000). Immunologically, MV was found to be capable of suppressing immune responses (McChesney and Oldstone, 1989; Tsujimura *et al.*, 1998). Recent studies have suggested that MV infects and alters functions of T cells (Fugier-Vivier *et al.*, 1997; Hahm *et al.*, 2004; Niewiesk *et al.*, 2000) and antigen-presenting cells (APC) (Grosjean *et al.*, 1997;

Schnorr *et al.*, 1997; Servet-Delprat *et al.*, 2000). This infection skews the T-cell response to a Th2 phenotype (Griffin and Ward, 1993). MV generates type I interferon (IFN) that acts via a signal transducer and an activator of a transcription (STAT) 2-dependent, but STAT1-independent, pathway (Hahm *et al.*, 2005). Thus, it is possible that the MV contributes in autism by suppressing immune function.

#### IV. Role of Environmental Factors in Autistic Development

Occupational and/or environmental exposure to mercury is believed to harm human health possibly through modulation of immune homeostasis (Lawrence and McCabe, 2002). Several studies have demonstrated that imbalances in immune regulation by metals can lead to inadequate or excessive production of inflammatory cytokines (Gilmore, 2003; Croonenberghs *et al.*, 2002; Safieh-Garabedian *et al.*, 2004). Alternatively, metals can lead to inappropriate activation of lymphoid subsets involved in acquired immunity to specific antigens. Some resultant pathologies may include chronic inflammatory processes and autoimmune diseases. Metals may change the response repertoire by direct and indirect means by influencing expression of new antigens, new peptides, and/or may change antigen presentation by modifying the antigen-presenting complex (Lawrence and McCabe, 2002).

##### A. MERCURY LINK WITH AUTISM

Exposure to methyl mercury (MeHg) in high doses has profound effects on the CNS and can be fatal. Neuropathological studies indicate that the occipital cortex and cerebellum are most affected. Prenatal exposure studies from Japan and Iraq demonstrated diffused CNS damage with disruption of cellular migration (Bernard *et al.*, 2000; Choi, 1989). It has been hypothesized that postnatal exposure to thimerosal, a mercurial preservative added to the vaccines, may be associated with autism and learning/speech disorders. However, no direct test of this association has yet been reported (Tager-Flusberg *et al.*, 2000). No human studies as yet document any adverse effects of prenatal or early postnatal exposure to elemental mercury or mercury vapor (Davidson *et al.*, 2004).

A study by Vahter *et al.* (2000) examined the different species of mercury in the blood of pregnant women. They found high correlations between inorganic mercury levels in blood and urine during early pregnancy, a significant correlation between cord and maternal blood, and decreased mercury levels during

lactation—presumably the result of excretion in milk. The fetal brain is especially susceptible to damage from exposure to organic mercury.

Astrocytic swelling, excitatory amino acid (EAA) release and uptake inhibition, as well as EAA transporter expression inhibition, are known sequelae of MeHg exposure. The presence of Hg causes an inability of astrocytes to maintain control of the proper milieu of the extracellular fluid and, in turn leads to neuronal demise (Shanker *et al.*, 2003).

Heavy metals have been shown to exert immunotoxic effects on humoral immunity as well. IgG3 production is most sensitive to inhibition by mercuric chloride (HgCl<sub>2</sub>) followed by IgG1 and IgG2b and then IgM and IgG2a. HgCl<sub>2</sub> exerts early, inhibitory effects on B-cell activation. This is manifested by the inhibition of RNA, DNA, and antibody synthesis. (Daum *et al.*, 1993). Metals by binding to SH radicals in proteins and other such groups, can cause autoimmunity by modifying proteins which via T cells activate B cells that target the altered proteins fibrillar, a 34-kDa protein component of many small nucleolar ribonucleoprotein particles inducing autoimmunity. They also cause aberrant MHC II expression on altered target cells (Hu *et al.*, 1997a, b; Hultman *et al.*, 1994; Pollard *et al.*, 1997). Thimerosal (ethyl mercury) in individuals with pre-disposing HLA molecules bind to CD26 or CD69 and induce antibodies against these molecules (Vojdani *et al.*, 2003). Furthermore, the CD95/Fas apoptotic signaling pathway that is of critical importance in regulating peripheral tolerance, is disrupted by low and environmentally relevant concentrations of Hg<sup>2+</sup> (McCabe *et al.*, 2005).

## V. Inflammatory Mediators in Autism

Inflammation has an important repairing function, but, in CNS, frequently is the cause of damage. Usually neuroinflammation has the tendency to succumb to damage, which would explain the CNS pathology associated with autism (Chavarria and Alcocer-Varela, 2004).

The various components involved in the inflammatory response in the CNS include the participation of different cellular types of the immune system (macrophages, mast cells, T and B lymphocytes, dendritic cells), resident cells of the CNS (microglia, astrocytes, neurons), adhesion molecules, complement proteins, cytokines, and chemokines among other proteic components. Chemotaxis plays an important role in the recruitment of cells to the CNS. The lymphocyte recruitment implies the presence of chemokines and chemokine receptors, the expression of adhesion molecules, the interaction between lymphocytes and the bloodbrain barrier (BBB) endothelium, and their passage through the BBB to arrive at the site of inflammation (Little *et al.*, 2002). Recent studies by Vargas

*et al.*, 2005) demonstrated that the two cells involved in inflammation are microglia and astroglia, which are essential for many neuronal functions. The presence of activated cells in their samples suggests that there is a chronic and a sustained neurological damage in autism. This activation could lead to abnormal function of neurons and synapses. Absence of any lymphocytes (T cells), plasma cells/antibody in the brain parenchyma suggests only the activation of astrocytes and microglial cells. These two cells are the classical players of innate immune response and thus demonstrated that an adaptive immunity is not playing an active role in autism. However, one cannot rule out the possibility of adaptive immunity playing a prominent role early in the prenatal or postnatal development phase of autism.

#### A. PROINFLAMMATORY CYTOKINES AND CHEMOKINES IN AUTISM

As previously discussed, several lines of evidence indicate the immune system can influence the normal activities of the CNS. Cells of the immune system are present in the CNS where they show increased chemical activities under conditions of inflammation and disease. Cytokines and chemokines, secreted by either immune or non-immune cells, play critical roles in many chronic and acute inflammatory conditions. Therefore, it is likely that mediators of immune cell-CNS interactions under normal conditions and in diseased states are skewed. Indeed, studies from several laboratories provide evidence for an altered/unique cytokine profile in autism. Two pro-inflammatory chemokines, macrophage chemoattractant protein-1 (MCP-1), thymus, activation-regulated chemokine (TARC), and an anti-inflammatory and modulatory cytokine, TGF- $\beta$ 1, were consistently elevated in the brain regions studied (Vargas *et al.*, 2005). MCP-1, a chemokine involved in monocyte and T-cell activation for trafficking into areas of tissue injury, was elevated both in brain parenchyma and CSF in cytokine protein array studies (Vargas *et al.*, 2005). In that study, immunocytochemistry revealed that astrocytes had infiltrated the cerebellum and cerebral cortex. The increased expression of MCP-1 in autism implies that it is linked to microglial activation and perhaps also to the recruitment of additional macrophages and microglia to areas of the cerebellum (Vargas *et al.*, 2005). There is evidence that MCP-1 may serve a signaling function in the damaged CNS that is distinct from its role in proinflammatory events (Little *et al.*, 2002). Its role in autism is not clear but its presence signifies inflammatory insult (Perrin *et al.*, 2005) or neuronal survival and protection (Uicker *et al.*, 2005). The observation that human fetal glial cells and their progenitors express specific receptors for chemokines and can be stimulated to produce MCP-1 as well as proliferate in response to chemokines, supports a role for these cytokines as regulatory factors during ontogeny (Rezaie and Male, 1999). MCP-1 expression in the cerebellum during prenatal

development suggests an association with maturation of Purkinje cells (Meng *et al.*, 1999). Like MHC-class II expression in microglia during CNS modeling, MCP-1 elevation in the brain of autistic patients may reflect persistent fetal patterns of brain development.

Other cytokines with pro-inflammatory and anti-inflammatory effect were also increased in the brain of patients with autism (Vargas *et al.*, 2005). An example of anti-inflammatory cytokines is TGF- $\beta$ 1, a key anti-inflammatory cytokine involved in tissue remodeling following injury. Upregulating extracellular matrix proteins accomplish this. It can suppress specific immune responses by inhibiting T-cell proliferation and maturation while downregulating MHC class II expression especially in the brain stem (Johns *et al.*, 1992). In the study of Vargas *et al.* (2005), immunocytochemical studies, TGF- $\beta$ 1 was localized mostly within reactive astrocytes and neurons in the cerebellum. Purkinje cells that exhibited microscopic features of degeneration showed marked reactivity for TGF- $\beta$ 1. The elevation of this cytokine in autism may reflect a compensation mechanism to diminish neuroinflammation or remodel and repair injured tissue.

The prominent inflammatory cytokine profile was repeated in cerebrospinal fluid (CSF) as well in patients with autism (Vargas *et al.*, 2005). The marked increase of MCP-1 in CSF is indicative of pro-inflammatory pathway activation in the brain of autistic patients. This may be associated with activation of microglial cells as seen in brain parenchyma studies. These studies indicate that cytokine activation plays an important role in immune mediated processes and that their presence in the CSF in autistic patients may reflect an ongoing stage of inflammatory reactions. These reactions are associated with neuroglial activation and/or neuronal injury. The persistent elevation of cytokines in CSF also might reflect a neurodevelopmental arrest, as some of the cytokines are normally elevated during phases of neurodevelopment. Elevated levels of IL-6 are also observed in diseases associated with developmental disorders. IL-6 and ciliary neurotrophic factor (CNTF), a neuronal growth factor, share the same intracellular receptor. This suggests that IL-6 may influence the nervous system via pathways normally used by growth factors. Relatively little is known about the effect of IL-6 or chemokines on CNS neurons, the transduction mechanism linked to IL-6 or chemokine receptors, the pathways involved in IL-6, or chemokine induced neuropathology (Nelson *et al.*, 2004). In a small sample, Gupta and his colleagues (1998) found that tumor necrosis factor-alpha (TNF- $\alpha$ ), another potent proinflammatory cytokine, was significantly increased in autistic populations. This finding was further corroborated in a study of Jyonouchi and collaborators (2001) who tested 71 autistic children aged 2–14 years and compared them with healthy siblings and other controls. In this study, innate immune responsiveness showed that in 59 of 71 (83.1%) autistic patients, lipopolysaccharide (lps) activated peripheral blood mononuclear cells (PBMCs) produced

levels of TNF- $\alpha$ , IL-1 $\beta$ , and/or IL-6 that were greater than 2 SD above the control mean (CM) values. Without stimulus, the basal level of proinflammatory/counter-regulatory cytokines was high in autistic patients. With stimulants phytohemagglutinin (PHA), tetanus, IL-12p70, and IL-18 of adaptive immunity PBMCs from 47.9 to 60% of autistic patients produced greater than 2 SD above the CM values of TNF- $\alpha$  depending on stimulants. The investigators concluded that a majority of the autistic children in their group exhibited excessive or poorly regulated innate immune responses especially involving increased TNF- $\alpha$  (Jyonouchi *et al.*, 2001). It should also be noted that although NO has been known to exert neuroprotective effects at low to moderate concentrations, NO becomes neurotoxic as the concentration increases. Excessive NO production can cause oxidative stress to neurons, ultimately impairing neuronal function and resulting in neuronal cell death (Abbott and Nahm, 2004). Indeed, plasma NO is high in some children with autism. This elevation may be related to IFN- $\gamma$  activity (Sweeten *et al.*, 2004).

Taken together these studies suggest that several chemokines/cytokines and/or inflammatory mediators are involved in the pathogenesis of autism. However, their exact cellular source or mechanism of actions remains to be the subject of further investigations.

## VI. Involvement of Toll-Like Receptors (TLRs) in Autism

The inflammatory signaling cascades leading to c-fos activation in glial cells have shown that activation by LPS in glial cells occurs via the serum response element (SRE) or cyclic AMP/calcium response element (CRE) in an independent manner, and involves the Elk1 or CREB/ATF-1 transcription factors. Elk1-mediated transactivation was dependent on p38 mitogen-activated protein kinase (MAPK), suggesting a crucial role of these factors in mediating inflammatory responses in the CNS (Simi *et al.*, 2005).

Additionally, Ozato *et al.* (2002) described the response of cell-surface toll-like receptors (TLRs) upon binding to microbial pathogens. There are at least 10 TLRs that recognize ligands from bacteria, viruses, yeast, and nucleic acids from viruses as well. There is a high binding specificity of the different TLRs for each microbial structure referred to as pathogen-associated molecular patterns (PAMPs) (Ozato *et al.*, 2002). The best studied is TLR4 that binds LPS from gram-negative bacteria. The ligation of LPS to cell surface TLR4 initiates a signal cascade that results in the activation of intracellular nuclear factor kappa beta (NF $\kappa$ B) and the transcription of numerous genes involved in immune responses. This signaling pathway appears to be common to all the TLRs whether the PAMPs originate from bacteria, virus, or yeast.

The central nervous system exhibits a similar immune reaction to pathogenic infection. There is a broad expression of TLRs in human brain astrocytes, oligodendrocytes, and microglia (Bsibsi *et al.*, 2002). Astrocytes and oligodendrocytes express mRNA for TLR2 that recognizes fungal, gram-positive, mycobacterial components and TLR3 that recognize, double-stranded RNA. Microglia cells express mRNA for a wide range of TLR family members (TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, and TLR9) much like other cells of the monocytic lineage (Bsibsi *et al.*, 2002). The binding of LPS to TLR on microglia cells (brain macrophage) leads to the innate expression of cytokines, chemokines, extracellular matrix proteins, proteolytic enzymes, and complement proteins in the brain parenchyma (Aloisi, 2001; Nguyen *et al.*, 2002). It is also well established that glial cells participate in innate immune responses in human CNS (Nguyen *et al.*, 2002). The sharing of the TLR receptors between the astrocyte and microglia is another example where the neurology is communicating with immunology using common molecules. Microglial cells are the resident macrophage-like population in the CNS. Microglial cells remain quiescent until injury or infection activates the cells to perform effector inflammatory and antigen presenting cell (APC) functions. Mouse microglial cells express mRNA for all of the recently identified TLRs, TLR1–9. Furthermore, stimulation of quiescent microglia with various TLR agonists, including LPS (TLR4), peptidoglycan (TLR2), polyinosinic-polycytidylic acid (TLR3), and CpG DNA (TLR9) activated the cells to up-regulate unique patterns of innate and effector immune cytokines and chemokines at the mRNA and protein levels. In addition, TLR stimulation activated up-regulation of MHC class II and costimulatory molecules, enabling the microglia to efficiently present myelin Ags to CD4+ T cells. Thus, microglia appear to be a unique and important component of both the innate and adaptive immune response, providing the CNS with a means to rapidly and efficiently respond to a wide variety of pathogens (Olson and Miller, 2004).

## VII. Autoimmunity in Autism

Inflammation has been linked with autoimmune insult. Aberrant innate immune response against endotoxin and immune reactivity to dietary proteins may be associated with apparent dietary product associated gastrointestinal inflammation in autistic children (Jyonouchi *et al.*, 2002, 2005). Another piece of information is the virus-induced autoimmune response to developing brain myelin that may impair anatomical development of neural pathways in autistic children (Singh *et al.*, 1993). The consequent anatomical changes of such autoimmune reactions could impair the nerve-impulse transmission and ultimately lead to life-long disturbances of higher mental functions (such as

learning, memory, communication, social interaction, etc.) that are seen in autistic populations.

#### A. MATERNAL ANTIBODIES CAN TRIGGER THE ATTACK IN AUTISM

Conceptually, it is possible that IgG from the mother can pass through the placental barrier and can react with antigenic proteins expressed on cell surface of lymphoid and/or neuronal tissues of the fetus and result in neuronal cell death. Since antigens expressed on lymphocytes are found on cells of the central nervous system and, perhaps, on other tissues of the developing embryo, it has been suggested that aberrant maternal immunity may be associated with the development of autism (Warren *et al.*, 1990). In fact, there is evidence in the literature supporting the importance of maternal antibodies in autism. Dalton and his colleagues (2003) have shown that serum antibodies that bind to rodent Purkinje cells and other neurons were detectable in a mother of three children: the first normal, the second with autism, and the third with a severe specific language disorder. The same serum when injected into pregnant mice during gestation produced altered exploration and motor coordination and changes in cerebellar magnetic resonance spectroscopy in the mouse offspring.

#### B. MMR VACCINATION MAY INCREASE RISK VIA AN AUTOIMMUNE MECHANISM

As previously mentioned, antibodies from autistic patients against MBP and neuron-axon filament protein (NAFP) cross-reacts with anti-measles antibody and human herpes-6 antibody (Singh *et al.*, 1998). This observation supports the hypothesis that a virus-induced autoimmune response may play a causal role in autism (Singh, 2000). Seventy-five of 125 (60%) autistic sera specifically detected measles hemagglutinin (HA) protein of measles-mumps-rubella (MMR) and over 90% of MMR antibody-positive autistic sera were also positive for MBP autoantibodies, suggesting a strong association between MMR and CNS autoimmunity in autism (Singh *et al.*, 2002). In another study, Singh and Jensen (2003) showed that there were elevated levels of measles antibodies in autistic children with no reaction to mumps or rubella.

There is some evidence that autism arises shortly after immunization with measles-mumps-rubella (MMR) and/or diphtheria-pertussis-tetanus (DPT) vaccines (Megson, 2000). Antibody levels to three vaccines, MMR, DPT, and DT (diphtheria-tetanus), were measured and it was found that the level of MMR antibodies was significantly higher in autistic children as compared to normal children (Singh *et al.*, 2002). There was a very high degree of specificity for MMR antibodies, particularly for measles (Singh *et al.*, 2002). The same result was also

found when monovalent measles vaccine was used instead of the trivalent MMR vaccine, furthermore pointing to a problem of only the measles subunit (Singh and Jensen, 2003). A high positive correlation (90% or greater) between the MMR antibody and the MBP autoantibody (Singh *et al.*, 2002) was detected. The deduction drawn from these studies is that the measles subunit of the MMR vaccine triggers an autoimmune reaction in a significant number of autistic children (Singh, 2000; Singh and Jensen, 2003; Singh *et al.*, 2002). MMR vaccine seems to induce interferon-gamma (IFN $\gamma$ ) only in breast-fed infants after primary measles immunization, a Th-1 cellular response. These results imply that the feeding pattern of infants can have a long-term effect on the immune modulation beyond weaning (Pabst *et al.*, 1991).

### C. POTENTIAL LINKAGE OF ENVIRONMENTAL FACTORS WITH AUTOIMMUNE EVENTS IN AUTISM

Autoantibodies (primarily IgG) to neuronal cytoskeletal proteins, neurofilaments (NFs), MBP, were prevalent in male workers exposed to mercury. These findings were confirmed in rats and mice. There were significant correlations between IgG titers and subclinical deficits in sensorimotor function. Thus, peripheral autoantibodies to neuronal proteins are predictive of neurotoxicity, since histopathological findings were associated with disease damage. There was also evidence of astrogliosis (indicative of neuronal CNS damage) and the presence of IgG concentrated along the blood brain barrier (El-Fawal *et al.*, 1999). Autoimmune response to mercury has also been shown by the transient presence of antinuclear antibodies (ANA) and antinucleolar antibodies (ANoLA) (Fagala and Wigg, 1992; Hu *et al.*, 1997; Nielsen and Hultman, 1999).

In an interesting study with newborns and thimerosal, autoimmune disease-sensitive mice were compared to strains resistant to autoimmunity. Mice were injected solely with thimerosal, a thimerosal-vaccine combination, or a saline solution. The comparative study showed growth delay, reduced locomotion, exaggerated response to novelty, and densely packed, hyperchromic hippocampal neurons with altered glutamate receptors and transporters in autoimmune mice. These animal studies implicate that impaired immunity might put some children at risk of developing autism after being exposed to thimerosal (Chian and Lipkin, 2004).

## VIII. Summary

The immunopathogenesis of autism is presented schematically in Fig. 1. Two main immune dysfunctions in autism are immune regulation involving

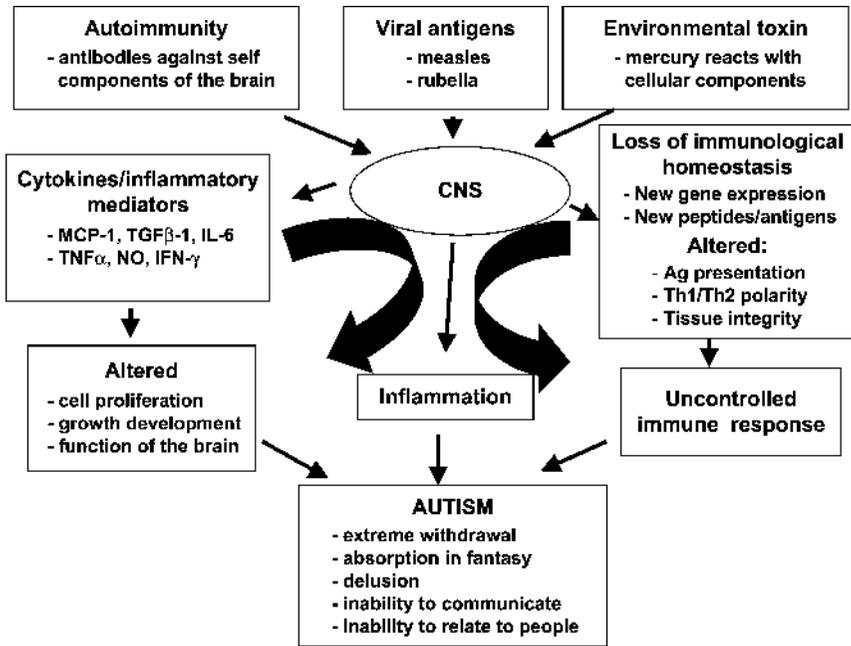


FIG. 1. Schematic presentation of immunopathogenesis of autism.

pro-inflammatory cytokines and autoimmunity. Mercury and an infectious agent like the measles virus are currently two main candidate environmental triggers for immune dysfunction in autism. Genetically immune dysfunction in autism involves the MHC region, as this is an immunologic gene cluster whose gene products are Class I, II, and III molecules. Class I and II molecules are associated with antigen presentation.

The antigen in virus infection initiated by the virus particle itself while the cytokine production and inflammatory mediators are due to the response to the putative antigen in question. The cell-mediated immunity is impaired as evidenced by low numbers of CD4 cells and a concomitant T-cell polarity with an imbalance of Th1/Th2 subsets toward Th2. Impaired humoral immunity on the other hand is evidenced by decreased IgA causing poor gut protection. Studies showing elevated brain specific antibodies in autism support an autoimmune mechanism. Viruses may initiate the process but the subsequent activation of cytokines is the damaging factor associated with autism. Virus specific antibodies associated with measles virus have been demonstrated in autistic subjects.

Environmental exposure to mercury is believed to harm human health possibly through modulation of immune homeostasis. A mercury link with the immune system has been postulated due to the involvement of postnatal exposure

to thimerosal, a preservative added in the MMR vaccines. The occupational hazard exposure to mercury causes edema in astrocytes and, at the molecular level, the CD95/Fas apoptotic signaling pathway is disrupted by  $Hg^{2+}$ . Inflammatory mediators in autism usually involve activation of astrocytes and microglial cells. Proinflammatory chemokines (MCP-1 and TARC), and an anti-inflammatory and modulatory cytokine, TGF- $\beta$ 1, are consistently elevated in autistic brains. In measles virus infection, it has been postulated that there is immune suppression by inhibiting T-cell proliferation and maturation and downregulation MHC class II expression. Cytokine alteration of TNF- $\alpha$  is increased in autistic populations. Toll-like-receptors are also involved in autistic development. High NO levels are associated with autism. Maternal antibodies may trigger autism as a mechanism of autoimmunity. MMR vaccination may increase risk for autism via an autoimmune mechanism in autism. MMR antibodies are significantly higher in autistic children as compared to normal children, supporting a role of MMR in autism. Autoantibodies (IgG isotype) to neuron-axon filament protein (NAFP) and glial fibrillary acidic protein (GFAP) are significantly increased in autistic patients (Singh *et al.*, 1997). Increase in Th2 may explain the increased autoimmunity, such as the findings of antibodies to MBP and neuronal axonal filaments in the brain. There is further evidence that there are other participants in the autoimmune phenomenon. (Kozlovskaja *et al.*, 2000). The possibility of its involvement in autism cannot be ruled out. Further investigations at immunological, cellular, molecular, and genetic levels will allow researchers to continue to unravel the immunopathogenic mechanisms' associated with autistic processes in the developing brain. This may open up new avenues for prevention and/or cure of this devastating neurodevelopmental disorder.

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