Cytokine dysregulation in autism spectrum disorders (ASD): Possible role of the environment

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1. Introduction

Autism spectrum disorders (ASD) are neurodevelopmental diseases that affect an alarming number of individuals. The etiological basis of ASD is unclear, and evidence suggests it involves both genetic and environmental factors. There are many reports of cytokine imbalances in ASD. These imbalances could have a pathogenic role, or they may be markers of underlying genetic and environmental influences. Cytokines act primarily as mediators of immunological activity but they also have significant interactions with the nervous system. They participate in normal neural development and function, and inappropriate activity can have a variety of neurological implications. It is therefore possible that cytokine dysregulation contributes directly to neural dysfunction in ASD. Further, cytokine profiles change dramatically in the face of infection, disease, and toxic exposures. Imbalances in cytokines may represent an immune response to environmental contributors to ASD. The following review is presented in two main parts. First, we discuss select cytokines implicated in ASD, including IL-1β, IL-6, IL-4, IFN-γ, and TGF-β, and focus on their role in the nervous system. Second, we explore several neurotoxic environmental factors that may be involved in the disorders, and focus on their immunological impacts. This review represents an emerging model that recognizes the importance of both genetic and environmental factors in ASD etiology. We propose that the immune system provides critical clues regarding the nature of the gene by environment interactions that underlie ASD pathophysiology.

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key facilitators of cross-systemic communication. Cytokines are proteins that control the nature, duration, and intensity of an immune response. They are highly pleiotropic, and can act in an autocrine, paracrine, and/or endocrine fashions. Immune cells, including dendritic cells, macrophages, neutrophils, T cells, and B cells, are the primary source of cytokines; though many additional cell types, including neurons, produce and respond to them. Cytokines share structural similarities and signaling pathways with neurotrophins and neurologically relevant growth factors. In many ways, cytokines represent a common language between the immune system and the nervous system.

Cytokines influence both the development and function of the nervous system. Their significance varies based on the timing, duration, and intensity of the neuro-immune interaction. For example, cytokines impact the developing brain differently than the adult brain; and may be beneficial at one concentration while harmful at another. Cytokines are involved in normal aspects of neurodevelopment, including progenitor cell differentiation, cellular localization/migration within the nervous system, and synaptic network formation (Deverman and Patterson, 2009). During infection and illness, cytokines mediate neurological changes associated with fever and sickness behavior by signaling directly to the hypothalamus (Dantzer, 2001; Skurlova et al., 2006). Emerging evidence also implicates cytokines in higher order neurological functions, including cognition and memory (McAfoose and Baune, 2009; Derecki et al., 2010). Imbalanced cytokine production, signaling, and/or regulation can therefore have a wide range of neurological consequences.

2. Cytokines in ASD

Aberrant expression of cytokines and their signaling intermediaries is often noted in ASD (Table 1). This is observed in the brain (Vargas et al., 2005; Grigorenko et al., 2008; Voineagu et al., 2011; Zats and Rennert, 2011) peripheral blood (Molloy et al., 2006; Ashwood et al., 2011a, 2011b) and the gastrointestinal tract (Defelice et al., 2003; Ashwood et al., 2004). Cytokine imbalances during development and/or throughout life could impact neural activity and mediate behavioral aspects of the disorder. The following considers the significance of several cytokines linked to ASD.

2.1. Interleukin (IL)-1B

IL-1B is an inflammatory cytokine expressed very early in immune responses (Jiang et al., 1997). In tissue, IL-1B propagates inflammation by activating local immune cells and the vascular endothelium. Systemically, IL-1B stimulates IL-6 production and eventually an acute phase response in the liver. Systemic IL-1B can cross the blood brain barrier (Banks et al., 1991) and stimulate its own expression in the hypothalamus, which leads to neuroendocrine changes associated with fever and sickness behavior (Dantzer, 2001; Skurlova et al., 2006). IL-1B receptors are structurally related to toll-like receptors (TLRs), and signaling is achieved through NF-κB and MAP kinase (MAPK) signaling cascades (O’Neill, 2000). IL-1B belongs to an evolutionarily conserved family of proteins that function beyond immunity (Barkesy et al., 2007). It shares structural homology with fibroblast growth factors (Zhang et al., 1991), which are critical in embryonic neurodevelopment, and are implicated in autism and schizophrenia (Tabares-Seisdedos and Rubenstein, 2009; Stevens et al., 2010).

Genes for IL-1B, its receptor, and its receptor-associated proteins are associated with intellectual disability, schizophrenia, and autism (Katila et al., 1999; Piton et al., 2008; Handley et al., 2010). Children and adults with autism have increased plasma IL-1B and skewed cellular IL-1B responses following stimulation (Ashwood et al., 2011a, 2011b; Suzuki et al., 2011). Compared to controls, monocytes from children with ASD produce excessive IL-1B following LPS exposure (Jyonouchi et al., 2011).

Table 1

Cytokines in autism spectrum disorders. A variety of independent clinical studies have linked cytokines to ASD. This table presents detailed findings for each individual cytokine. Often multiple cytokines were associated with ASD in a single study, which is noted in parentheses.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Findings in autism</th>
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<td>IL-1β</td>
<td>Elevated plasma levels in children with ASD, correlated with regressive onset. (IL-6, IL-8 and IL-12p40 also elevated)</td>
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<td></td>
<td>Elevated plasma levels in high functioning children with ASD. (IL-1RA, IL-5, IL-8, IL-12p70, IL-13, IL-17 and GRO-α also elevated)</td>
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<td>Elevated plasma levels in adults with severe ASD. (IL-6 and endotoxin levels also elevated)</td>
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<td>Peripheral blood cells from ASD subjects produce higher baseline levels. (Similar trends for IL-6 and TNF-α)</td>
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<td></td>
<td>Peripheral blood cells from ASD subjects produce higher levels with TLR2 or TLR4 stimulation, and lower levels with TLR-9 stimulation. (Similar trends for IL-6 and TNFα)</td>
<td>(Entstrom et al., 2010)</td>
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<td>IL-6</td>
<td>Elevated plasma levels in children with ASD, correlated with regressive onset. (IL-1β, IL-8, and IL-12p40 also elevated)</td>
<td>(Ashwood et al., 2011a, 2011b)</td>
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<td>Lymphoblasts from ASD subjects produce more IL-6. (Also TNF-α)</td>
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<td>Increased IL-6 in postmortem brain stem specimens (various regions) from ASD subjects. (Also increased TGF-β and inflammatory chemokines)</td>
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<td></td>
<td>Increased IL-6 in postmortem brain tissue from ASD subjects. (Also increased TNF-α, IFN-γ, GM-CSF, and IL-8)</td>
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<td>Elevated IL-6 in postmortem brain tissue from mothers giving birth to a child with ASD. (Also IL-5 and IFN-γ)</td>
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<td>IL-4</td>
<td>Increased IL-4 in mid-gestational serum samples from mothers giving birth to a child with ASD (Also IL-5 and IFN-γ)</td>
<td>(Abdallah et al., 2011)</td>
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<td>Increased IL-4 in amniotic fluid samples from mothers giving birth to a child with ASD</td>
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<td>IFN-γ</td>
<td>Peripheral blood cells from ASD subjects stimulated with PMA-ionomycin were more likely to be IL-4+ (And less likely to be IFN-γ+)</td>
<td>(Gupta et al., 1998)</td>
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<td></td>
<td>Increased IFN-γ in mid-gestational serum samples from mothers giving birth to a child with ASD. (Also IL-4 and IL-5)</td>
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<td>Peripheral blood cells stimulated with PMA-ionomycin are less likely to be IFN-γ+ (And more likely to be IL-4+)</td>
<td>(Croonenberghs et al., 2002)</td>
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<td></td>
<td>Unstimulated whole blood from ASD subjects produced significantly more IFN-γ compared to controls. (Also increased IL-1RA, IL-6, and TNF-α)</td>
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<td></td>
<td>NK cells from children with ASD produced higher IFN-γ under resting conditions, and lower levels after stimulation. (Also observed with perforin and granzyme B)</td>
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<td>TGF-β</td>
<td>Increased IFN-γ in post mortem brain specimens from ASD subjects. (Also increased TNF-α, IL-6, GM-CSF, and IL-8)</td>
<td>(Ashwood et al., 2008)</td>
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<td></td>
<td>Decreased plasma TGF-β in adults with ASD. (Also IL-6 and inflammatory chemokines)</td>
<td>(Okada et al., 2007)</td>
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<td></td>
<td>Increased TGF-β levels in postmortem brain specimens (various regions) from ASD subjects. (Also IL-6 and inflammatory chemokines)</td>
<td>(Vargas et al., 2005)</td>
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2001; Enstrom et al., 2010), and lower levels following exposure to TLR 9 agonists (Enstrom et al., 2010). The IL-1 antagonist, IL-1ra, is also increased among ASD subjects (Suzuki et al., 2011). IL-1ra reduces inflammation by competing for the IL-1 receptor, and increased levels may represent an attempt to counteract inflammation in ASD. Postmortem brains from ASD subjects had normal IL-1B levels (Li et al., 2009), but given that peripheral IL-1B can enter the brain (Banks et al., 1991), increased systemic levels could directly impact neurological processes.

IL-1B disruption can have a variety of neurological consequences relevant to ASD. The cytokine and its receptors are found throughout the nervous system during critical developmental periods (Giulian et al., 1988). IL-1B induces neural progenitor cell proliferation in some CNS regions, while inhibiting it in others (de la Mano et al., 2007). This could contribute to the region-specific overgrowth and undergrowth observed in the ASD brain. Excessive synapse formation is partially mediated by the IL-1 receptor and receptor-associated proteins (Yoshida et al., 2011). Altering these proteins can tip the balance between excitatory and inhibitory signaling, which might underlie neurological features of ASD (Rubenstein and Merzenich, 2003). Increased IL-1ra in ASD suggests an attempt to counterbalance IL-1B and may or may not be beneficial. Following brain injury, IL-1ra upregulation serves a neuroprotective role by dampening excessive inflammation (Lodick and Rothwell, 1996). However, if administered during critical windows of neurodevelopment, IL-1ra can negatively impact neurogenesis, brain morphology, memory consolidation, and behavior (Spulber et al., 2008, 2010, 2011). This shows that some level of IL-1B signaling is essential during development. In adulthood, IL-1B is implicated in CNS disorders like Alzheimer’s disease and the advancement of amyloid-containing plaques (Griffin et al., 1995). While excessive IL-1B contributes to pathology in some cases, it may have a protective role in others. For example, IL-1B limits neuronal damage following excitotoxic exposures (Strijbos and Rothwell, 1995), and mice lacking IL-1B fail to undergo remyelination following experimental autoimmune encephalitis (EAE) induction (Mason et al., 2001). IL-1B is involved in higher order brain processes and is induced in the hippocampus during learning processes, and is critical for maintenance of long-term potentiation (LTP) (Ross et al., 2003). Both over expression (Barrientos et al., 2009) and under expression of IL-1 beta (Goshen et al., 2007; Labrousse et al., 2009) are associated with impairments in memory and learning.

In summary, IL-1B participates in neurological processes, and appears to have a role in both CNS pathology and healing. Normal, homeostatic levels of IL-1B and its antagonist IL-1ra are necessary for proper brain development and function. This “Goldilocks” state is typical of many cytokines, where too much or too little is not desirable. Alterations in IL-1B systems due to genetic mechanisms or environmental exposures may contribute to autism.

2.2. Interleukin (IL)-6

IL-6 is an inflammatory cytokine that shares functional properties with IL-1B. Like IL-1B, IL-6 is produced early in immune reactions, although it appears later and persists longer (Jiang et al., 1997). IL-6 is best known for stimulating the acute phase response in the liver, generating fever, and activating lymphocytes. Despite the functional similarities with IL-1B, IL-6 differs drastically in terms of structure and signaling properties. It is a member of the neuroepoietic cytokine family, which includes leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), and IL-11. These cytokines signal through a gp130 receptor complex (Ward et al., 1994), and activate JAK-STAT (specifically STAT 3) and MAPK signaling pathways (Heinrich et al., 2003). In addition to their inflammatory properties, neuroepoietic cytokines have a number of well-described roles in the nervous system, and are intricately involved in neurodevelopment and function (Bauer et al., 2007; Mcafoose and Baune, 2009). IL-6 and its receptors are expressed at low levels in the healthy brain (Gadient and Otten, 1994a, 1994b) and at higher levels in a variety of disease states (Huell et al., 1995; Hang et al., 2004). Peripheral IL-6 can cross the blood brain barrier and influence a variety of processes in the adult brain (Banks et al., 1994).

Prenatal cytokine imbalances may contribute to neurodevelopmental disorders like autism and schizophrenia through “fetal programming”. Fetal programming is the concept that maternal factors like inflammation and chronic stress can alter the gestational environment, skew development, and lead to long term physiological and behavioral consequences (Patterson, 2009; Bilbo, 2010). IL-6 readily crosses the placenta and enters fetal tissues, which is unique among cytokines, and can induce changes in placental physiology and gene expression (Zaretsky et al., 2004; Aaltonen et al., 2005; Dahlgren et al., 2006; Hsiao and Patterson, 2011). Animal models show that IL-6 is necessary and sufficient to alter neurodevelopmental outcomes, leading to changes in behavior, cognition, neuropathology, GABA dysregulation, and skewed immune function among offspring (Samuelsson et al., 2006; Smith et al., 2007). Similar effects are seen with prenatal exposure to infection or the infectious mimic poly I:C (Smith et al., 2007; Malkova et al., 2012). IL-6 can impact a variety of processes in the developing brain. IL-6 and its family members regulate self-renewal among neuronal precursors (Escary et al., 1993; Yoshimatsu et al., 2006), direct neuronal migration (Wei et al., 2011), promote cell survival (Kushima et al., 1992), and regulate neurite outgrowth (Ihara et al., 1997). IL-6 exposure during critical windows can also alter synaptic networks. Chronic IL-6 overexpression reduces expression of glutamate receptors and L-type calcium channels in culture and in vivo (Vereyken et al., 2007), and increases the ratio of excitatory to inhibitory synapses in cultures of cerebellar granular cell cultures (Wei et al., 2011). This is of particular interest in autism, given that skewed excitatory and inhibitory ratios may be an underlying factor in its pathogenesis.

Despite intriguing evidence from animal models, two recent human studies question whether gestational IL-6 alone contributes to autism. A retrospective examination of IL-6 in archived mid-pregnancy maternal serum and amniotic fluid showed that increased levels associated with developmental disorders, but not autism (Abdallah et al. 2011; Goines et al., 2011b). This suggests that gestational IL-6 might be a marker for neurodevelopmental diseases, but is insufficient on its own to cause ASD. IL-6 can affect many sequential phases of neurodevelopment, so the timing of the exposure will largely dictate the neurological outcome. Therefore, excessive IL-6 during one phase of neurodevelopment could have one set of consequences, while similar expression during another phase has an entirely different effect. A longitudinal examination of IL-6 throughout gestation is therefore needed to obtain a more complete picture of its relevance in neurodevelopmental disorders.

IL-6 can also impact processes in the adult brain, and physiological levels are critical for homeostasis, cognition, learning, and memory. Physiological levels of IL-6 are critical for normal CNS function, and both over and under expression leads to neurological problems. Mice overexpressing IL-6 in the CNS have overt symptoms including tremor, ataxia, and seizure (Campbell et al., 1993), and more subtle alterations in cognition and avoidance behaviors (Heyser et al., 1997). IL-6 is transcribed in the hippocampus during LTP (Balschun et al., 2004). Overexpression of IL-6 reduces LTP (Bellinger et al., 1995; Li et al., 1997), while under expression increases it and improves learning and memory (Balschun et al., 2004; Braida et al., 2004). With regard to social behaviors, mice overexpressing IL-6 are more social than mice that lack the cytokine, while mice lacking IL-6 demonstrate higher aggression and emotionality (Alleva et al., 1998; Armario et al., 1998).

Many independent studies show IL-6 dysregulation in individuals with autism. Children and adults with the disorder have higher circulating IL-6 levels compared to typically developing controls (Emanuele et al., 2010; Ashwood et al., 2011a, 2011b). Further, cellular IL-6 production is increased with and without stimulation (Jyonouchi et al., 2001; Enstrom et al., 2010; Malik et al., 2011). Increased IL-6 is also found in postmortem brain specimens from ASD subjects. Specifically, immunohistochemical analysis of cerebellar sections showed significantly more IL-6 staining in autism postmortem brain specimens (Wei et al., 2011).
Two additional analyses of homogenates of the frontal cortex and anterior cingulate gyrus also showed higher IL-6 levels (Vargas et al., 2005; Li et al., 2009). Given the ability of IL-6 to impact processes in the adult brain, it is conceivable that increased IL-6 in autism could contribute to ongoing aspects of the disorder. Alternatively, it might be an epiphenomenon, and represent a biomarker of infectious or toxic environmental exposures and altered biological homeostasis.

In summary, there is extensive evidence that IL-6 can alter neurodevelopment and function. While it is unclear whether gestational IL-6 in humans is related to autism, a dysregulation of IL-6 is observed later in life in individuals with autism. The significance of these findings is unclear, and may be the result of other genetic and environmental factors in autism. These possibilities warrant further investigation.

2.3. Interleukin (IL)-4

IL-4 is a class I cytokine that activates Jak/Stat (STAT6), MAPK, and PI3 kinase signaling cascades (Nelms et al., 1999). Immunologically, IL-4 has a variety of interesting roles, and can 1) induce “alternatively activated” macrophages that promote tissue repair over inflammation, 2) activate basophils and mast cells, 3) promote B-cell isotype switching towards IgG1 and IgE, 4) participate in immune responses against helminthes by inducing epithelial cell turnover in the gut, and 5) participate in allergy and asthma-related immune responses (Kuperman and Schleimer, 2008; Byers and Holtzman, 2011; Oliphant et al., 2011).

The receptors for IL-4 are expressed in the brain under normal conditions throughout life (Nolan et al., 2005). During development, IL-4 promotes oligodendrogenesis among neuronal progenitor cells, (Bouktyvskiy et al., 2006), and improves survival in embryonic hippocampal cultures (Araujo and Cotman, 1993). IL-4 influences retinal circuitry by regulating progenitor cell proliferation and differentiation (da Silva et al., 2008). During later phases of neurodevelopment, IL-4 can alter synapse formation; increasing the proportion of GABAergic synapses in cell culture models (Sholl-Franco et al., 2002).

Two recent studies have linked developmental IL-4 exposures to autism, though its role in pathogenicity versus protection is unclear. Mothers giving birth to a child with autism show higher levels of IL-4 in mid-pregnancy serum samples (Goines et al., 2011b) and amniotic fluid (Abdallah et al., 2011) compared to controls. IL-4 is not thought to cross the placenta, and maternal serum and amniotic fluid IL-4 may or may not relate to IL-4 in fetal tissues. Other cytokines were also upregulated in these archived samples, including IFN-γ, TNF-α, and the anti-inflammatory cytokine IL-10. This raises the question of whether IL-4 acts alone, or in concert with other cytokines. Increased IL-4 may represent a regulatory reflex to inflammation along with IL-10. IL-4’s role in pregnancy and fetal health is unclear. Increased levels during pregnancy have been associated with poor outcomes such as preterm labor (Dudley et al., 1996) but also healthy outcomes such as protection from preeclampsia (Kronborg et al., 2011; Rajakumar et al., 2011). More subtle neurodevelopmental outcomes have not thoroughly been explored with respect to gestational IL-4.

In the adult brain, IL-4 largely serves a neuroprotective role, and is associated with higher order cognitive processes. It is upregulated during CNS inflammation, inducing alternative activation of glial cells and protecting from apoptosis (Garg et al., 2009; Sholl-Franco et al., 2009; You et al., 2011). In a mouse model for Alzheimer’s disease, IL-4 can attenuate disease progression (Kiyota et al., 2010). Following LPS exposure, IL-4 reduces inflammation and improves memory and LTP in the aged hippocampus (Nolan et al., 2005). An elegant study by Derecki et al. showed that IL-4-producing T cells accumulate in the meningeal spaces during cognitive tasks. Depletion of IL-4 led to an inflammatory phenotype among meningeal myeloid cells, and a dramatic decline in cognitive capacity. Remarkably, cognitive deficits in IL-4 deficient mice could be reversed by reintroducing the cytokine in adulthood (Derecki et al., 2010). Among individuals diagnosed with autism, plasma and CNS IL-4 levels appear to be normal (Vargas et al., 2005; Li et al., 2009; Ashwood et al., 2011a, 2011b). However, IL-4 producing T cells are proportionately higher in children with autism compared to controls (Gupta et al., 1998). Given the evidence that meningeal IL-4 producing T cells are critical for normal cognitive function in adulthood, it is possible that dysregulation in this cell population could contribute to altered behavior throughout life (Derecki et al., 2010).

Collectively, IL-4 serves a variety of neurological roles, and is increased in autism. Its role during gestation is unclear due to a dearth of in-vivo studies of pregnancy and neurobehavioral outcomes following developmental IL-4 exposures. The significance of increased IL-4 producing T cells in subjects with autism is also unclear. Extensive evidence suggests that IL-4 is neurologically beneficial, so it may be that increased IL-4 in autism represents an immunological attempt to regulate other detrimental processes, and does not contribute to the disease itself. Future studies should explore this possibility.

2.4. Interferon-gamma (IFN-γ)

Interferon gamma (IFN-γ) is the sole type II interferon. It shares some functional similarities with type I interferons like IFN-α and IFN-β but has unique structural features, receptors, and signaling pathways. IFN-γ is produced primarily by T cells and Natural Killer (NK) cells during cell-mediated immune responses, and functions largely to activate macrophages and combat viral infections (Boehm et al., 1997; Schroder et al., 2004). It signals mainly through the JAK/STAT (STAT1), and MAPK cascades (Hu et al., 2001; Platianis, 2005). IFN-γ and IL-4 counterbalance one another’s activity via TnT/TnT2 interactions, so dysregulation in one cytokine often impacts the other. It is therefore not surprising that both cytokines are implicated in ASD.

Developmental exposure to IFN-γ has been linked to autism. Mothers of children with autism have higher serum IFN-γ during the second trimester compared to controls (Goines et al., 2011b). Like IL-4, IFN-γ does not cross the placenta, and the relationship between maternal serum levels and fetal exposure to the cytokine is unclear. If the cytokine is present in fetal tissues, it could interfere with normal neural development and synapse formation. IFN-γ promotes neuronal differentiation among neuronal progenitor cells (Barish et al., 1991; Jonakait et al., 1994; Wong et al., 2004; Bouktyvskiy et al., 2006; Zahir et al., 2009; Leipzig et al., 2010; Li et al., 2010), however, these cells appear to be abnormal and exhibit compromised function and strange patterns of neuronal marker expression (Walter et al., 2011). IFN-γ also impacts dendritic morphology and synapse formation, leading to long-term changes in cellular connectivity and communication. Depending on cell culture conditions, IFN-γ either promotes or inhibits dendrite outgrowth through STAT1 and MAPK signaling pathways (Barish et al., 1991; Kim et al., 2002a; Wong et al., 2004; Song et al., 2005; Andres et al., 2008). In culture, excessive IFN-γ alters patterns of excitatory signaling and receptor expression (Vikman et al., 2001), and animals lacking the cytokine have fewer pre-synaptic terminals (Victorio et al., 2010). Interestingly, mice overexpressing IFN-γ show increased MHC I in the brain (Corbin et al., 1996). MHC I is critical for T cell and NK cell recognition of self and foreign entities, and was historically thought to be absent in the CNS. However, recent studies have demonstrated that it is expressed in the CNS, and has an essential role in synapse formation and plasticity (Shatz, 2009). IFN-γ may therefore induce abnormalities in synaptic organization by altering MHC I expression. Collectively, these studies show that direct exposure to IFN-γ can cause abnormal neurodevelopment, which may explain features of autism.

If excess IFN-γ is not present in fetal tissues, excessive maternal levels could have an indirect impact on fetal development. Interestingly, IFN-γ has a variety of critical roles in pregnancy, and directs aspects of placental development, health, and maintenance (Murphy et al., 2009). Increased gestational IFN-γ is associated with adverse.

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pregnancy outcomes including recurrent miscarriage (Jenkins et al., 2000). Therefore, IFN-γ might be an indicator of compromised health in pregnancy, which could lead to neurodevelopmental abnormalities.

Peripheral IFN-γ is up-regulated in a number of neurological disorders including multiple sclerosis (Martins et al., 2011) and Down’s syndrome (Torre et al., 1995). Individuals with autism have increased plasma levels of IFN-γ (Singh, 1996), which correlates with other peripheral inflammatory mediators such as nitric oxide (Sweeten et al., 2004a, 2004b). Peripheral immune cells from ASD subjects produce higher basal levels of IFN-γ but fail to respond further following immunological stimulation (Gupta et al., 1998; Croonenberghs et al., 2002; Enstrom et al., 2009a). In addition to peripheral IFN-γ dysregulation, postmortem brain specimens showed increased levels of IFN-γ (Li et al., 2009), suggesting IFN-γ may directly impact CNS processes in autism. In the developed nervous system, IFN-γ is historically associated with neurodegeneration, although some evidence suggests it may have a beneficial role. IFN-γ can cross the blood brain barrier at low levels (Pan et al., 1997), but is barely detectable in the healthy nervous system (Traugott and Lebon, 1988; De Simone et al., 1998). In the CNS, IFN-γ is up-regulated following infectious exposures (De Simone et al., 1998), and in diseases including cerebral palsy (Folkther et al., 2004), multiple sclerosis (Traugott and Lebon, 1988); HIV dementia (Nolting et al., 2009), and Parkinson’s (Barcia et al., 2011; Mangano et al., 2011). High levels are harmful in cell culture, and cause enhanced glutamate induced neurotoxicity (Mizuno et al., 2008). However, in cell culture and in vivo, low IFN-γ levels reduce oxidative stress-induced apoptosis through activation of astrocytes (Garg et al., 2009; Victorio et al., 2010) and may be neuroprotective in some cases. In a mouse model of Alzheimer’s disease, overexpressing IFN-γ actually attenuated plaque formation (Chakraborty et al., 2010), while protective roles for IFN-γ have been suggested during some phases of demyelinating autoimmune disorders (Kumar and Sercarz, 1998). It is therefore difficult to determine whether IFN-γ has a pathogenic role in ASD, or if it represents a potentially beneficial immune response to damage associated with genetic and/or environmental influences.

2.5. Transforming growth factor-beta (TGF-B)

TGF-B is a highly pleotropic cytokine that maintains immune homeostasis, directs lymphocyte differentiation, and orchestrates aspects of embryonic development. TGF-B is largely immunosuppressive; limiting excessive T cell activity and inflammation (Mantel and Schmidt-Weber, 2011). It exists in three isoforms, each with distinct and overlapping roles. TGF-B1 is best characterized, and is the founding member of the TGF-B superfamily of proteins, which includes growth differentiation factors, bone morphogenic proteins (BMPs), activins, and inhibins (Kingsley, 1994). TGF-B superfamily signaling occurs largely via SMAD pathways, though MAPK cascades are also triggered (Yu et al., 2002; Shi and Massague, 2003).

TGF-B superfamily proteins are critical for proper neurodevelopment. For example, BMPs have an important role in early neural induction and differentiation (Reissmann et al., 1996; Bachiller et al., 2000; Tropepe et al., 2001). TGF-B1 is involved in neuronal migration, survival, and synapse formation. Mice lacking the cytokine demonstrate improper CNS development, including a disorganized extracellular matrix, widespread neuronal degeneration, microgliosis, reduced expression of synaptophysin, and deficits in both glutamatergic and GABAergic synapses (Brionne et al., 2003; Heuvel et al., 2008; Vashlishan et al., 2008). Overexpressing TGF-B in vivo also disrupts the extracellular matrix, and leads to seizures, motor incoordination, hydrocephalus, and behavioral abnormalities (Wyss-Coray et al., 1995; Depino et al., 2011). Changes in CNS expression levels of IL-6, Neureglin 3 and reelin are also observed with TGF-B overexpression (Depino et al., 2011), which is intriguing because many of these proteins are altered in autism (Persico et al., 2001; Laumonnier et al., 2004; Fatemi et al., 2005). Interestingly, CNS overexpression of TGF-B during development vs. adulthood leads to opposite behavioral consequences (Depino et al., 2011). Early in life, overexpression led to decreased social behavior and heightened anxiety/depression behaviors, while overexpression later in life had the exact opposite effect. This highlights the importance of timing when considering the neurological consequences of cytokine imbalances.

There is no evidence for TGF-B dysregulation during gestational development in autism. This does not negate the possibility that it is involved, as these endpoints are extremely difficult to measure in vivo. However, there is evidence for TGF-B dysregulation in individuals diagnosed with the disorder themselves. Plasma TGF-B is decreased in children and adults with ASD (Okada et al., 2007; Ashwood et al., 2008), and lower levels of the cytokine correlate with more severe autism behaviors (Ashwood et al., 2008). The connection between low TGF-B and behavioral phenotype is unclear, although this finding lends promise to the goal of developing a simple ASD testing regime based on biological markers in addition to behavioral symptoms. In contrast to peripheral TGF-B in ASD, postmortem brain specimens show increased levels compared to controls (Vargas et al., 2005). The reason for this periphery brain disparity is unclear. Although, TGF-B does not cross the blood brain barrier (Kastin et al., 2003), the cytokine and its receptors are expressed normally throughout the nervous system (Gomes et al., 2005) and it is conceivable that peripheral and brain levels are independent. In the adult brain, TGF-B is generally thought to be neuroprotective. CNS levels spike following injury and infection, and increase with age, HIV dementia, and Alzheimer’s disease, which leads to protection against disease-related neural degeneration and apoptosis (Henrich-Noack et al., 1994; Krupinski et al., 1996; Ruocco et al., 1999; Buckwalter and Wyss-Coray, 2004; Doyle et al., 2010). However, TGF-B can also contribute to pathogenicity. For example, high levels of the cytokine cause glial scarring and fibrosis (Moon and Fawcett, 2001), and increase disease susceptibility and severity in a murine model of multiple sclerosis (Wyss-Coray et al., 1997).

Overall, the role of TGF-B in the nervous system is complex, and varies based on the timing and context of the interaction. Increased TGF-B in the brain of ASD subjects might represent a protective reflex to a disease state, or perhaps contribute to the pathology of the disease itself. In the periphery, ASD subjects have decreased TGF-B as well as increased inflammatory markers (Jyonouchi et al., 2001; Croonenberghs et al., 2002; Emanuele et al., 2010; Ashwood et al., 2011a, 2011b). This suggests a global immune dysregulation in ASD, with an improper balance between regulation and activation, which could have wide reaching consequences for many systems in the body.

3. Environmental factors in autism and immune dysfunction

There is now general consensus that autism has an environmental component (Pessah, 2008; Hallmayer et al., 2011). For our purposes, the “environment” is a broad term used to define non-genetic, toxic, infectious, and/or immune factors that may contribute to the disorder. The hypothesis follows that an ill-timed exposure could cause autism in genetically susceptible individuals (Fig. 1). For example, some genes associated with autism may cause inappropriate immune responses (Heuer et al., 2011; Onore et al., 2011), while others reduce the capacity to deal appropriately with toxins (D’Amelio et al., 2005). These individuals are more susceptible to environmental influences, which could lead to ASD.

Environmental toxicants can cause both neural and immune dysfunction. This is likely mediated through disrupted cell signaling and homeostasis. Many toxicants alter calcium homeostasis, which can have a variety of consequences for immune development and function (Limke et al., 2003; Toscano and Guillart, 2005; Savignac et al., 2007; Vig and Kinet, 2009; Pessah et al., 2010; Bhatti et al., 2011). Toxicants can also disrupt endocrine function, which can have a variety of immunological consequences (Rivest, 2010; De Vito et al., 2011; Schug et al., 2011). The following section considers environmental factors that...
may be related to autism, and focuses on their role in the immune system.

3.1. Heavy metals

Heavy metals like lead and mercury are widespread environmental toxins. Developmental exposure to these compounds is associated with lower IQ, endocrine disruptions, and behavioral disturbances (Bellinger and Dietrich, 1994; Steuerwald et al., 2000; Cordier et al., 2002; Selevan et al., 2003; Winnike, 2011). Heavy metals also have immunotoxic properties, leading in some instances to autoantibody production (Waterman et al., 1994; Bagenstose et al., 1999; Rowley and Monestier, 2005) and skewed cytokine profiles (discussed below). Both of these immunological phenomena are observed in ASD (Enstrom et al., 2009b; Onore et al., 2011). These features have made them possible, though controversial, candidates in autism.

3.2. Heavy metals: lead

Lead has a variety of toxic mechanisms, and shares structural features with calcium which allows it to compete for binding sites (Toscano and Guilarte, 2005). In addition to its neurotoxic activity, lead is highly immunotoxic (Toscano and Guilarte, 2005; Mishra, 2009). At high levels, it is immunosuppressive, leading to increased production of regulatory cytokines and enhanced susceptibility to infection (Valentino et al., 2002). One study showed that lead can tip the balance between inflammation and regulation by increasing expression of IFN-γ and reducing TGF-β (Goebel et al., 2000): a cytokine profile which has been observed in autism (Singh, 1996; Gupta et al., 1998; Croonenberghs et al., 2002; Ashwood et al., 2008; Enstrom et al., 2009a; Li et al., 2009). Lead and pro-inflammatory cytokines can function in concert to alter the nervous system. When co-administered to glial cells, lead and cytokines such as IL-1β changed matrix metalloproteinase expression in a manner that was not observed when either was administered alone (Lahate et al., 2002) and suggests that immune and environmental factors could act synergistically on tissue remodeling.

There are conflicting reports regarding lead in autism. Higher serum lead levels have been documented in a few studies (Cohen et al., 1976; Shannon and Graef, 1996; Filippek et al., 1999), although more recent studies show no difference between autism and control populations (Tian et al., 2011; Albizzati et al., 2012). Polymorphisms in ALAD, a gene associated with heavy metal toxicity, have been described in children with autism and controls (Stamova et al., 2011; Tian et al., 2011). Mercury loads correlated with the expression of several immunologically relevant genes across all study participants. Further, there were some unique correlations in the autism group for genes involved in antigen presentation and recognition of self. This suggests that individuals with ASD may have a unique immunological susceptibility to heavy metals, but the significance of these findings is not clear. Although genomic expression profiles may suggest correlational changes in autism,
analysis of common polymorphisms associated with mercury transport and excretion, namely MT1a, DMT1, LAT1, and MTF1, were unable to detect any differences in autism (Owens et al., 2011). However, in contrast, a different study showed that polymorphisms in MTF1 and the heavy metal transport gene (SLC11A3) are associated with the ASD (Seraje et al., 2004). Additional genetic analyses are needed to rectify these disparate findings.

3.4. Pesticides

Pesticides are fairly non-persistent toxic compounds that are deliberately spread throughout the environment in mass quantities. While minimizing off-target toxicity is a primary goal in pesticide development, several products have been banned once their toxic potential in humans was recognized. Developmental exposure to several types of pesticides, including organophosphates (OPs), organochlorines (OCs), and pyrethroids, is associated with neurodevelopmental dysfunction and an increased risk for ASD (Garry et al., 2002; Kamel and Hoppin, 2004; Eskenazi et al., 2007; Roberts et al., 2007; Eskenazi et al., 2010; Bouchard et al., 2011). Genetic analyses also suggest that individuals with ASD may be less capable of excreting pesticides, due to expression of a less-active variant of the OP-metabolizing enzyme paraoxonase (D’Amelio et al., 2005; Pasca et al., 2006). In addition to their neurotoxicity, many pesticides impact the immune system and cytokine production, which may be relevant for ASD (Banerjee et al., 1996; Li, 2007).

3.5. Pesticides: organochlorines

Organochlorine (OC) pesticides are structurally and functionally variable toxic compounds that include members like hexachlorobenzene, dioxin, and DDT; many of which have been banned (Crinion, 2009). OCs interfere with calcium signaling, voltage sensitive sodium channels, and GABA receptors, leading to neuro- and immunotoxicity (Casida, 2009; Crinion, 2009; Heusinkveld and Westerink, 2012). Immunologically, these compounds impact both humoral and cell-mediated processes, and reduce the host response to infectious challenges (Banerjee et al., 1996; Reed et al., 2004; Nagayama et al., 2007). A handful of studies have explored the impact of OCs on cytokine profiles. DDT reduced IL-2 production in cell culture by interfering with the transcription factor NF-κB (Ndebele et al., 2004). Given the central role of NF-κB in cytokine production and function, this is likely to have a wide-ranging immunological impact. In contrast, cases of DDT or lindane poisoning in humans is associated with increased serum levels of IL-2, IL-4, and TNF-alpha, as well as decreased levels of IFN-γ (Daniel et al., 2002; Seth et al., 2005). It is not clear why IL-2 production in response to OCs differs in cell culture versus in vivo. However, the findings of increased IL-4 and decreased IFN-γ in humans suggest a Th2 immune bias following OC exposure, which could have downstream consequences for allergic and asthmatic disorders. Similar immune profiles have been reported in some studies of autism (Gupta et al., 1998).

3.6. Pesticides: organophosphates

Organophosphates (OP) are esters of phosphoric acid that were introduced as replacements for various banned OC pesticides. They act to terminate acetylcholinesterase (AChE) inhibition, leading to altered cholinergic signaling, parasympathetic and sympathetic perturbations, seizures, and/or respiratory arrest. OPs can also be toxic in the absence of AChE inhibition, and may induce higher order neural and cognitive dysfunction (Duyser et al., 2001; Costa, 2006; Pancetti et al., 2007). Some OP developmental effects are more severe in males than females (Levin et al., 2001, 2010), which is intriguing given that autism also has a heavy male bias (Baron-Cohen et al., 2005).

OPs induce a variety of immunological phenomena relevant to autism (Galloway and Handy, 2003; Li, 2007). In general, OPs appear to induce a prolonged inflammatory state that may evolve into an adaptive response characterized by up-regulation of both Th1 and Th2 cytokines. Acute OP intoxication is related to system-wide production of inflammatory mediators (Hamaguchi et al., 2006; Roeyen et al., 2008; Anand et al., 2009). Within the CNS, acute and chronic exposure to OPs results in increased inflammatory cytokines including IL-1β and IL-6 in multiple brain regions (Svensson et al., 2001; Henderson et al., 2002; Williams et al., 2003; Dhote et al., 2007; Dillman et al., 2009; Johnson and Kan, 2010); similar to findings in the autism brain (Vargas et al., 2005; Li et al., 2009). OP induced inflammation can be long term, re-emerging and persisting long after the initial exposure (Chapman et al., 2006). In primary cultures of human peripheral blood cells or astrocytes, the OP pesticide chlorpyrifos up-regulates IL-6 and IFN-γ production and the expression of related genes (Mense et al., 2006). Children born to mothers working in agriculture had higher production of Th2 cells at 12 and 24 months of age. (Duramad et al., 2006a, 2006b). An immunosuppressive response can also be induced following exposure to various OPs, perhaps as a reflex to the toxin’s initial immunotoxic properties (Williams et al., 2003; Damodaran et al., 2006; Dhote et al., 2007). Collectively, these data suggest that OPs impact cytokine profiles in the short and long term, increasing inflammatory cytokines, Th1 and Th2 profiles and compensatory regulatory activity.

3.7. Pesticides: pyrethroids

Pyrethroids are a group of insecticides and repellants derived from natural compounds in the Chrysanthemum genus of plants. They mediate their toxicity by disrupting calcium signaling, interfering with voltage-sensitive sodium channels, and inducing oxidative stress (Shafer et al., 2005; Soderlund, 2012). Exposure to these compounds is associated with a wide range of neurodevelopmental problems in mammalian models (Shafer et al., 2005; Wolansky and Harrill, 2008). Immunological abnormalities are also linked to pyrethroids. In human peripheral blood mononuclear cells, exposure to different pyrethroid compounds suppressed both IFN-γ and IL-4 expression in a time and concentration dependent manner (Diel et al., 2003). In a monocytic cell line, various synthetic pyrethroids and their metabolites reduced expression of immunoregulatory IL-10, and increased production of more inflammatory cytokines IL-12 and TNF-α (Zhang et al., 2010). In a Xenopus laevis model, application of environmentally relevant concentrations of various pyrethroids increased IL-1β expression (Martini et al., 2010). In primary human fetal astrocytes, the pyrethroid pesticide cyfluthrin was found to have an activating effect, and increased the expression of genes involved in IFN-γ and IL-6 production and signaling (Mense et al., 2006).

3.8. Halogenated aromatic hydrocarbons

Halogenated aromatic hydrocarbons are toxic compounds that are highly resistant to degradation. Two examples are polychlorinated biphenyls (PCBs) and polylbrominated diphenyl ethers (PBDEs). PCBs and PBDEs consist of two aromatic rings with various chlorine (PCBs) or bromine (PBDEs) substitutions. There are over two hundred different congeners of each PCBs and PBDEs, which differ based on the number and orientation of halogen substitutions. Toxicity in this class of molecules is congener-specific, and involves varying degrees of 1) interaction with the Aryl hydrocarbon receptor (Ahr) (Mitchell and Elferink, 2009; Gu et al., 2012), 2) disruption of endocrine systems (Morse et al., 1993; Van Birgelen et al., 1995; Stoker et al., 2005; Kuriyama et al., 2007; Lema et al., 2008), and/or 3) interference with calcium homeostasis through interactions with the ryanodine receptor (Coburn et al., 2008; Pessah et al., 2009, 2010; Kim et al., 2011; Langelund et al., 2012). These toxic mechanisms have both neuro- and immunological significance.

Children with ASD may be uniquely susceptible to halogenated aromatic hydrocarbons. Postmortem analysis showed altered ryanodine receptor expression in the brain of autism subjects compared to
controls, which could alter their sensitivity to Ryr-reactive compounds (Voineagu et al., 2011). Further, in a mouse model of Rett syndrome, a genetic disorder that shares features with autism, developmental exposure to PBDE-47 caused epigenetic, cognitive, and social differences that were not observed in wild type mice (Amir et al., 1999; Woods et al., 2012). Finally, children with ASD have unique immune responses to PBDEs, which is discussed in detail below (Ashwood et al., 2009).

3.9. Halogenated aromatic hydrocarbons: polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are ubiquitous in the environment and in animal and human tissues. They were widely used as additives to industrial oils and lubricants until the late 1970s when their adverse health effects became apparent. PCB exposure is linked to adverse pregnancy outcomes and neurobehavioral deficits (Kuratsune et al., 1971; Rogan et al., 1988; Chen and Hsu, 1994; Eriksson and Fredriksson, 1998; Howard et al., 2003; Kenet et al., 2007; Lein et al., 2007; Tsukimori et al., 2008; Kim et al., 2009; Boix et al., 2010; Kim and Pessah, 2011), as well as immune dysfunction. In studies involving marine animals, murine models, and humans, PCBs lead to generalized immune suppression, characterized by diminished cellular immunity and atrophied lymphoid organs (Davis and Safe, 1990; Narayanan et al., 1998; Fournier et al., 2000; Shin et al., 2000; Tan et al., 2003; Beineke et al., 2005; Lejs et al., 2009). Cytokine profiles are also impacted by PCBs. In a murine model, perinatal exposure to a PCB mixture induced inflammatory cytokine expression (primarily IL-6) in the brain of adult offspring (Hayley et al., 2011). In human blood cells, PCB 52 and PCB 133 induced transcriptional changes in several cytokine signaling and regulation pathways (Wens et al., 2011). This effect was only observed in cells from male donors, suggesting a male-bias for some PCB effects. Another study showed that PCB 118 enhanced IL-4 producing T-cell development (Gaspar-Ramirez et al., 2012), which correlates with findings in children with ASD and their mothers (Gupta et al., 1998; Abdallah et al., 2011; Goines et al., 2011b).

PCBs likely exert their immunotoxicity by interfering with immunologically-relevant signaling pathways. For example, dioxin-like PCBs interfere with Ahr signaling, which is critical for maintaining a healthy immune balance in the skin and gut (Li et al., 2011; Monte Leone et al., 2011). PCBs also disrupt the key cytokine signaling pathways JAK/STAT and MAPK. Immune cells exposed to PCB 47 and 153 have compromised function and altered STAT 5 and ERK phosphorylation (Canesi et al., 2003). STAT 5 activation is central for regulatory T cell development (Burchill et al., 2003; Adamson et al., 2009), and activation of this molecule by PCBs might mediate some of their immunosuppressive effects. Finally, some PCB congeners interfere with ryanodine receptors, which are expressed widely in the immune system. These receptors are regulated by cytokines including TGF-B (Hosoi et al., 2001; Pessah et al., 2010), and can induce IL-6 production after engagement (Treves et al., 2011). In summary, PCBs alter immune function through a variety of mechanisms that may be relevant to immune profiles observed in autism.

3.10. Halogenated aromatic hydrocarbons: polybrominated diphenyl ethers (PBDEs)

PBDEs are a group of flame retardants that are the subject of growing concern due to their structural and functional similarities to PCBs. They are widely dispersed in the environment and bioaccumulate up the food chain (Johnson-Restrepo and Kannan, 2009). Environmental PBDE levels have jumped dramatically in the last several decades, and are increasingly found in human tissues and breast milk (Noren and Meironyte, 2000; Darnerud et al., 2001). Of note, this heightened prevalence is concurrent with the apparent rise in ASD diagnoses (Hertz-Picciotto and Delwiche, 2005; Messer, 2010). While not linked specifically to ASD, PBDE exposure is associated with improper neurodevelopment, hormonal disruptions, and a variety of behavior, motor, and cognitive issues (Alm et al., 2008; He, He et al., 2008; Roze et al., 2009; Herbstman et al., 2010; Kodavanti et al., 2010; Schreiber et al., 2010; Dingemans et al., 2011).

Some evidence shows that PBDEs can impact immune activity. Studies involving marine and murine models link PBDEs to changes in the immune system, including thymic and splenic atrophy, increased production of IL-10, lymphocyte depletion, reduced antibody recall responses, and decreased responses to pathogens (Fowlkes et al., 1994; Thuwander, 1999; Beineke et al., 2005; Zhou et al., 2006; Beineke et al., 2007a, 2007b; Lundgren et al., 2009; Watanabe et al., 2010; Bondy et al., 2011; Fair et al., 2012). There is little data regarding the impact of PBDEs on cytokine production and signaling, and future studies should examine these endpoints in more detail. One recent study considered the effect of BDE-47 on cytokine responses in children with autism and controls. Peripheral blood mononuclear cells obtained from ASD and typically developing controls were pretreated with PBDE-47 and stimulated with LPS. Analysis of supernatant cytokines showed that BDE-47 had a divergent impact on cells from ASD versus typical controls. Among controls, BDE-47 caused a significant decrease in inflammatory cytokines production (IL-6, IL-12, GM-CSF, TNF-α), indicating broad immune suppression. In contrast, children with ASD only down-regulated IL-6 in the presence of BDE-47, and had significantly higher IL-1B responses (Ashwood et al., 2009). This suggests that BDE-47 can enhance some aspects of inflammation in ASD. This diagnosis-specific immunotoxic effect suggests that children with ASD respond differently to PBDEs than typical children. Robust inflammation in response to such exposures during critical developmental windows could have long term neurological effects, and may be a possible mechanism in ASD. Future studies should continue to examine a potential role for PBDEs in immune dysfunction and neurodevelopmental disorders like autism.

3.11. Pathogenic exposures in autism

Early-life infections can skew fetal development, leading to aberrant neural and immune activity. This is a widely suggested etiological mechanism in schizophrenia, and is increasingly implicated in autism as well (Patterson, 2009). Several infections, including measles, cytomegalovirus, and rubella during pre- and perinatal periods have been associated with autism (Chess, 1977; Markowitz, 1983; Ivarsson et al., 1990; Sweeten et al., 2004a, 2004b; Libbey et al., 2005; Meyer et al., 2011). A recent large-scale epidemiological study showed that infection-related hospitalizations during pregnancy significantly increased the risk of ASD (Atladottir et al., 2010). Interestingly, the risk was not associated with any specific type of infection, suggesting that the general immune mechanism controlling the response to the pathogen rather than the pathogen itself were involved. This response is likely guaranteed to include cytokines. Indeed, there is clinical evidence for altered gestational cytokine milieu in autism (Abdallah et al., 2011; Goines et al., 2011b), which could be related to infectious exposures, and may mediate aspects of the disorder.

As discussed in previous sections, several studies have linked early immune challenges to long-term changes in behavioral and immune parameters. For example, mice prenatally exposed to influenza (Fatemi et al., 2002a; Shi et al., 2003), the viral mimic Poly I:C (Vuillermot et al., 2010), or the bacterial component LPS (Romero et al., 2010) demonstrate long term neurological and behavioral abnormalities. This effect is largely mediated by cytokines like IL-6 and IL-1B (Samuelsson et al., 2006; Smith et al., 2007; Bilbo et al., 2008). Prenatal infectious exposures can also impact the developing immune system, and lead to long term immune dysregulation. This was demonstrated by increased levels of IL-6, IL-2, and TNF-α in adult animals that had been prenatally exposed to LPS (Romero et al., 2010). Another interesting set of studies showed that an early life infection led to...
neurological deficits in adulthood that only became apparent after an immunological stimulation (Bilbo et al., 2005a, 2005b; Bilbo and Schwarz, 2009). This suggests that early life infection can change immune responses later in life, and that this has neurological consequences. The concept that prenatal infections can impact both brain and immune development is intriguing, and should be explored further in autism.

Overall, when considering environmental exposures, it is important to take time to consider that the prevalence rates for ASD have increased dramatically over the last 10–20 years. These rates continue to increase year-on-year. Arguably concentrations for some compounds such as lead and chlorinated pesticides have fallen in the population since their removal or reduction from the environment in the 1970s. In contrast, levels of PBDEs and OPs have increased. Although it may be tempting to link compounds as risk factors for ASD based solely on similar time trends there is a need for more extensive research to begin to understand whether such temporal relationships are associated with risk for ASD. Future research should focus on the relationships between environmental exposures and risk for ASD diagnosis and whether environmental exposures to such compounds as PBDE induce cytokine responses that could modulate neuronal function in the pediatric population. Moreover, future research should discern whether children who develop a ASD are more sensitive to specific environmental exposures using cytokine production as readouts. More research focused on environmental exposures and ASD is warranted.

4. Conclusions

Cytokine imbalances are well documented in autism and have many interesting implications. Cytokines are intricately involved in neurodevelopment and neuronal function, and an ill-timed cytokine disruption can have long term neurological consequences. Further, cytokine expression is largely dependent on genetic and environmental influences. Therefore, they may represent a biomarker for genetic or environmental factors at play in autism. To illustrate the connection between immunity, genes, the environment, and neurodevelopmental outcome, consider two scenarios: First, an individual may be genetically poised to mount an inappropriate immune response to an infectious or toxic exposure. This individual might respond either too robustly or too weakly to resolve the threat without collateral damage to the brain and other body systems (including the immune system). Second, an individual may lack appropriate genetic machinery to excrete toxins; leading to toxic exposure. This individual might respond either too robustly or too weakly to mount an inappropriate immune response to an infectious or toxic exposure. This could lead to an amplification of the toxin's effects on a variety of body systems, including the brain and immune system. For each child, an environmental challenge during a critical window of development could have especially severe consequences, causing abnormal CNS function, altered immune phenotypes, and perhaps autism. These scenarios represent an emerging global view of autism that requires a broad consideration of several factors, including genes, the environment, and the immune system. Cross-disciplinary investigations that consider diverse biological contributions will be essential to untangle ASD.

Conflict of interest statement

The authors report no conflict of interest.

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